Göteborg 1981

ON THE SPECIES, TISSUE AND INDIVIDUAL DIVERSITY OF GLYCOSPHINGOLIPIDS

AND ON A ROLE OF HOST GLYCOLIPIDS IN AN INFECTIOUS PROCESS

Hakon Leffler

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41544702_0006

ON THE SPECIES, TISSUE AND INDIVIDUAL DIVERSITY OF GLYCOSPHINGOLIPIDS

AND ON A ROLE OF HOST GLYCOLIPIDS IN AN INFECTIOUS PROCESS

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Göteborgs Universitet kommer att offentligen försvaras i föreläsningssalen vid Institutionen för Medicinsk och Fysiologisk Kemi Lördagen den 19 December, 1981 kl.9.15 f.m.

av

Hakon Leffler

The thesis is based on the following reports:

I. Specific Pattern of Glycosphingolipids Enriched in a Mucosa Scraping of Human Small Intestine.
by K.-E. Falk, K.-A. Karlsson, H. Leffler and B.E. Samuelsson (1979) FEBS Letters 101,273-276.

II. Selected Ion Monitoring of Glycosphingolipid Mixtures. Identification of Several Blood Group Type Glycolipids in the Small Intastine of an Individual Rabbit.
by M.E. Breimer, G.C. Hansson, K.-A. Karlsson, H. Leffler, W. Pimlott and B.E. Samuelsson (1979) Biomedical Mass Spectrometry 6,231-241.

III. Blood Group Type Glycosphingolipids from Small Intestine of Different Animals Analysed by Mass Spectrometry and Thin-Layer Chromatography.A Note on Species Diversity.
by M.E. Breimer, G.C. Hansson, K.-A. Karlsson and H. Leffler (1981) J.Biochemistry(Tokyo) 90,589-609.

IV. Separation and Characterisation of Hematosides with Different Sialic Acids and Ceramides from Rat Small Intestine.Different Composition of Epithelial Cells versus Non-Epithelial Tissue and of Duodenum versus Jejunum-Ileum.
by J.Ångström, M.E. Breimer, I. Griph, G.C. Hansson, K.-A. Karlsson and H. Leffler (1981) J.Biochem.(Tokyo) 90,909-921.

V. Chemical Identification of a Glycosphingolipid Receptor for Escherichia coli Attaching to Human Urinary Tract Epithelial Cells and Agglutinating Human Erythrocytes.
H. Leffler and C. Svanborg-Eden (1980) FEMS Microbiology Letters 8, 127-134.

ABSTRACT

LEFFLER, H., On the Species, Tissue and Individual Diversity of Glycosphingolipids and on a Role of Host Glycolipids in an Infectious Process, p. 1-44, Department of Medical Biochemistry, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden (1981).

The glycosphingolipids comprise a family of compounds found at the cell surface and other parts of the cell. Acting as recognition markers at the cell surface (e.g. blood group antigens and other antigens, receptors for microorganisms) they may confer selectively to the interaction in-between cells and between cells and the environment (e.g. host-pathogen interaction in the urinary tract infection).

The main part of the present work was devoted to give a static description of the glycosphingolipid structures and compositions found in small intestine of various species and in different parts of small intestine. Another part (paper V) was testing of the role of glycosphingolipids as receptors for adhering bacteria in urinary tract infection.

Improvement of methodology included purification of acidic glycolipids (paper IV), mass spectrometry of mixtures of intact glycosphingolipids (paper II, III), interpretation of proton NMR spectra (paper IV) and an assay for the binding of bacteria to glycolipids (paper V).

The non-acid glycosphingolipid fractions of small intestine of man, cat, rabbit, mouse, two strains of rat, guinea-pig, hen and codfish, and urinary sediment of man were analysed by thin-layer chromatography and mass spectrometry (papers I-III, V). Testing of the fractions for immunological activity supplied stereochemical information and confirmed the presence of antigenic determinants as suggested by mass spectra (e.g. Blood group A,B and Le^a and Forssman antigens).

The results of the descriptive study may be summarised as follows: Extending the findings of previous studies it showed that small intestine is a rich source of glycosphingolipids. The pattern of glycolipids varies qualitatively for different species and also individuals (two rat strains), including both major and minor components. For each animal the pattern of epithelial cells was different from that of non-epithelial tissue.

When the results were put in the context of literature data some peculiar features of mammalian glycosphingolipid diversity were noted and tentatively called heterophil species and tissue variations (by analogy with heterophil antigens): 1. The presence of one glycosphingolipid in one tissue type of several species does not correlate with the degree of evolutionary relation between the species. 2. The distribution among tissues of one glycolipid varies considerably between species. These types of variations have several interesting implications for the discussion on glycosphingolipid evolution and function and also some technical implications.

The last part of the work (paper V) showed that a group of glycosphingolipids, the globo series, may act as receptors for the binding of uropathogenic E. coli to epithelial cells and erythrocytes. These glycolipids contain the saccharide Galα1→4Gal as an internal or terminal part. This may be the structure recognized by the bacteria possibly together with adjacent parts. Further studies showed that recognition of globoside is a common property of E. coli isolated from pyelonephritis, less common for strains from cystitis and rare for E. coli of other origin. These results opened the possibility of new studies on: 1. Individual variations in susceptibility to infection in relation to P blood group (as the globo series of glycolipids are also antigens of the P blood group system). 2. The diagnostic use of receptor analogues. 3. The therapeutic use of receptor analogues.

Species investigated in the present (man, cat, rabbit, mouse, two rat strains, guinea pig, hen, cod fish and fimbriated Escherichia coli) and earlier work (sea anemone and honey bee).

Department of Medical Biochemistry, University of Göteborg
Göteborg, Sweden

ON THE SPECIES, TISSUE AND INDIVIDUAL DIVERSITY OF GLYCOSPHINGOLIPIDS

AND ON A ROLE OF HOST GLYCOLIPIDS IN AN INFECTIOUS PROCESS

by

Hakon Leffler

Göteborg 1981

ABSTRACT

LEFFLER, H., On the Species, Tissue and Individual Diversity of Glycosphingolipids and on a Role of Host Glycolipids in an Infectious Process, p. 1-44, Department of Medical Biochemistry, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden (1981).

The glycosphingolipids comprise a family of compounds found at the cell surface and other parts of the cell. Acting as recognition markers at the cell surface (e.g. blood group antigens and other antigens, receptors for microorganisms) they may confer selectively to the interaction in-between cells and between cells and the environment (e.g. host-pathogen interaction in the urinary tract infection).

The main part of the present work was devoted to give a static description of the glycosphingolipid structures and compositions found in small intestine of various species and in different parts of small intestine. Another part (paper V) was testing of the role of glycosphingolipids as receptors for adhering bacteria in urinary tract infection.

Improvement of methodology included purification of acidic glycolipids (paper IV), mass spectrometry of mixtures of intact glycosphingolipids (paper II, III), interpretation of proton NMR spectra (paper IV) and an assay for the binding of bacteria to glycolipids (paper V).

The non-acid glycosphingolipid fractions of small intestine of man, cat, rabbit, mouse, two strains of rat, guinea-pig, hen and codfish, and urinary sediment of man were analysed by thin-layer chromatography and mass spectrometry (papers I-III, V). This allowed the interpretation of the saccharide chain length, sequence of sugar types (hexose, hexosamine, deoxyhexose) and ceramide composition for up to 9 glycolipids in one mixture, including a novel group of compounds, e.g. tetra--heptahexosylceramides. The structures could be assigned to different bands in the thin-layer chromatogram, giving a powerful visualisation of species variability. Testing of the fractions for immmunological activity supplied stereochemical information and confirmed the presence of antigenic determinants suggested by mass spectra (below). Different hematosides of rat small intestine were purified and the structure studied by mass spectrometry, proton NMR spectroscopy of the intact glycolipids and by degradative methods (paper IV).

The results of the descriptive study may be summarised as follows: Extending the findings of previous studies it showed that small intestine is a rich source of glycosphingolipids. The pattern of glycolipids varies qualitatively for different species and also individuals (two rat strains), including both major and minor components. For each animal the pattern of epithelial cells was different from that of non-epithelial tissue. The latter was shown by studies on isolated epithelial cells of man (paper I) and rat (paper IV) and inferred from the ceramide structure of the different glycolipids for the other species.

Thus, glycolipids of epithelial cells contained more hydroxylated ceramides (hydroxy fatty acid and trihydroxy base) than those of non-epithelial tissue (having non-hydroxy fatty acids and dihydroxy base). Most fucolipids were found in epithelial cells, e.g. blood group A substances in cat, rabbit and one rat strain, blood group B substances in rabbit. Mouse epithelial cells contained gangliotetraosylceramide as the major complex glycolipid. Monohexosylceramides were found for all species but the sugar type varied (Gal or Glc). Di-, tri-, tetra- and/or pentahexosylceramides were found in the epithelial cells of cat, rabbit, rat and guinea-pig. Forssman hapten and globoside (P antigen) like structures were found only in non-epithelial tissue of man, cat, mouse, rat, guinea-pig and hen. The study of paper IV showed that for hematosides of rat small intestine the sialic acid was the N-glycoloyl type in epithelial cells of jejunum-ileum but the N-acetyl type in epithelial cells of duodenum and in non-epithelial tissue along the whole small intestine.

The codfish intestine contained only small amounts of glycolipids. These were monohexosylceramide (major) and hexosamine containing tri-, tetra- and pentaglycosylceramides. Two of the latter contained a terminal dihexosamine residue as found in the Forssman antigen but the immunological activity was not tested.

Finally the descriptive part of paper V showed that epithelial cells of the human urinary tract (sediment) may contain a high concentration of blood group type fucolipids (Le^a active pentaglycosylceramide) as found in human small intestine, but also contain glycolipids not found in small intestinal epithelial cells.

When the results were put in the context of literature data some peculiar features of mammalian glycosphingolipid diversity were noted and tentatively called heterophil species and tissue variations (by analogy with heterophil antigens): 1. The presence of one glycosphingolipid in one tissue type of several species does not correlate with the degree of evolutionary relation between the species. 2. The distribution among tissues of one glycolipid varies considerably between species. These types of variations have several interesting implications for the discussion on glycosphingolipid evolution and function and also some technical implications.

The last part of the work (paper V) showed that a group of glycosphingolipids, the globo series, may act as receptors for the binding of uropathogenic E. coli to epithelial cells and erythrocytes. These glycolipids contain the saccharide Galα1$\rightarrow$4Gal as an internal or terminal part. This may be the structure recognized by the bacteria possibly together with adjacent parts. Further studies showed that recognition of globoside is a common property of E. coli isolated from pyelonephritis, less common for strains from cystitis and rare for E. coli of other origin. These results opened the possibility of new studies on: 1. Individual variations in susceptibility to infection in relation to P blood group (as the globo series of glycolipids are also antigens of the P blood group system). 2. The diagnostic use of receptor analogues. 3. The therapeutic use of receptor analogues.

Key words: Glycosphingolipids, blood group substance, small intestine, urinary tract, species diversity, tissue diversity, mass spectrometry, bacterial adhesion, urinary tract infection.
ISBN 91-7222-447-9

UNIV. LEIDEN BIBL.

The present thesis is based on the following reports, which are referred to in the text by their Roman numbers:

I. Specific Pattern of Glycosphingolipids Enriched in a Mucosa Scraping of Human Small Intestine
by K.-E. Falk, K.-A. Karlsson, H. Leffler and B.E. Samuelsson (1979) FEBS Letters 101, 273-276.

II. Selected Ion Monitoring of Glycosphingolipid Mixtures. Identification of Several Blood Group Type Glycolipids in the Small Intestine of an Individual Rabbit
by M.E. Breimer, G.C. Hansson, K.-A. Karlsson, H. Leffler, W. Pimlott and B.E. Samuelsson (1979) Biomedical Mass Spectrometry 6, 231-241.

III. Blood Group Type Glycosphingolipids from Small Intestine of Different Animals Analysed by Mass Spectrometry and Thin-Layer Chromatography. A Note on Species Diversity
by M.E. Breimer, G.C. Hansson, K.-A. Karlsson and H. Leffler (1981) J. Biochemistry (Tokyo) 90, 589-609.

IV. Separation and Characterization of Hematosides with Different Sialic Acids and Ceramides from Rat Small Intestine. Different Composition of Epithelial Cells versus Non-Epithelial Tissue and of Duodenum versus Jejunum-Ileum
by J. Ångström, M.E. Breimer, I. Griph, G.C. Hansson, K.-A. Karlsson and H. Leffler (1981) J. Biochem. (Tokyo) 90, 909-921.

V. Chemical Identification of a Glycosphingolipid Receptor for Escherichia coli Attaching to Human Urinary Tract Epithelial Cells and Agglutinating Human Erythrocytes
by H. Leffler and C. Svanborg-Edén (1980) FEMS Microbiology Letters 8, 127-134.

Figure on Cover:

Species investigated in the present and earlier work, i.e. Man (paper I), Rabbit (paper II), Cat, Mouse, Rat, Guinea-Pig, Hen, Cod-Fish (paper III), two strains of Rat (papers III and IV and Ref. 27), Sea Anemone (Ref. 157), Honey Bee (Karlander et al. (1972) Biochim. Biophys. Acta 270, 117-131) and fimbriated E. coli (paper V and Ref. 144).

Acknowledgements:

My thanks are due to a number of persons for inspiring me to scientific work; teaching of practical and theoretical tasks, patience and long-term planning; giving of encouragement; collaboration within and outside the group; keeping things in order; patience; technical help; typing help; keeping the lab clean and nice; more encouragement; financial support; home support; baby sitting; neighbourhood support and more patience.

Suffice it to mention by name my son Carl to whom together with his friends this thesis is dedicated for never failing inspiration, a good deal of patience and genuine new outlooks.

Research grants were from the Swedish Medical Research Council, no. 3967, the University of Göteborg and Svenska Sällskapet för Medicinsk Forskning.

CONTENTS (Page)

1. INTRODUCTION (1)

1.1 The glycosphingolipids (1)

2. THE PRESENT STUDY: CONTEXT AND APPROACH (5)

3. METHODOLOGY (6)

3.1 Extraction and fractionation (6)
3.2 Chemical analysis (6)
3.2.1 Mass spectrometry (8)

4. SPECIES, TISSUE AND IDNVIDIUAL DIVERSITY OF GLYCOSPHINGOLIPID STRUCTURES (10)

4.1 Description of glycolipid diversity (10)
4.1.1 Which glycosphingolipids and parts of glycosphingolipids are involved in the variations? (10)
4.1.2 Some characteristics and regularities of glycolipid carbohydrate chain diversity (12)
4.1.3 Variation of ceramide structure (14)
4.2 Some functional and technical implications of glycosphingolipid diversity (16)
4.2.1 The "heterophil" character of glycolipid diversity (16)
4.2.2 The evolution and expression of glycosphingolipid patterns (16)
4.2.3 Glycosphingolipid function (18)
4.2.4 Glycosphingolipids in embryogenesis (19)
4.2.5 Glycosphingolipids in tumours (20)
4.2.6 Glycosphingolipids as heterophil antigens (20)
4.2.7 Species variability of other complex carbohydrates (21)
4.2.8 Different species as sources of antigens and receptor molecules (21)

5. THE ROLE OF HOST GLYCOLIPIDS IN AN INFECTIOUS PROCESS (22)

5.1 Introduction (22)
5.2 Present work (22)
5.3 Further studies (24)
5.4 Practical implications (25)

6. APPENDIX: INTRODUCTORY SURVEY OF GLYCOSPHINGOLIPID STRUCTURE, OCCURRENCE AND ROLE IN CELL SURFACE RECOGNITION (27)

6.1 Glycolipid structures (27)
6.2 Where are glycosphingolipids found? (31)
6.3 What determines the glycosphingolipid pattern of a tissue? (31)
6.4 Cell surface recognition (32)
6.5 Glycolipids as cell surface recognition markers (33)

REFERENCES (35)

1. INTRODUCTION

To see a world in a grain of sand
and a heaven in a wild flower
hold infinity in the palm of your hand
and eternity in an hour

William Blake

Selectivity and individuality are among the characteristic features of the interplay between living organisms and between parts of the same organism. For example, one type of bacteria only infects a certain tissue of a certain animal, some women get urinary tract infections more easily than others, blood of one individual can be transfused only to certain other individuals, sperms of one animal can only fertilize eggs of the same species, the embryo develops to form specifically organized tissues of the animal, the nerve cells of the brain connect to a functional network, etc. One mechanism which is thought, or known, to be involved in these and other such phenomena is a process of "recognition" at cell surfaces (1-5).

A prerequisite to ever understand the mechanism for such phenomena is the knowledge of which components are present at the cell surface and what their chemical structures and properties are (compare Ref. 6) and how they differ between various cell types.

The present thesis is about glycosphingolipids, a class of chemically related compounds, found in a wide variety of natural sources, probably mostly at the surface of cells. The aim of the work was two-fold. From one point of view it was to give an improved understanding of the chemistry and biology of glycosphingolipids. From another point of view it was to give an improved understanding of the chemical mechanism for cell surface recognition in some cases where glycolipids are known to take part.

1.1. The glycosphingolipids

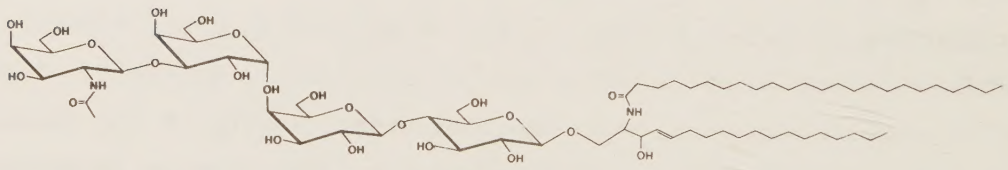
A glycosphingolipid consists of two parts (Fig. 1), a carbohydrate chain and a lipid part (6-12). Different glycosphingolipids have different carbohydrate chains and the large variety (71 structures are listed in Ref. 8 and about as many novel ones have been found since then) of glycolipids found in nature depends mainly on the highly variable carbohydrate chain. Thus, the carbohydrate chain may have different lengths, from one up to possibly sixty sugar residues (14-16). It may contain different carbohydrate residues and these may have different ring size and configuration and be linked together in different sequences and by different types of bonds.

The lipid part, ceramide, contains a fatty acid and a long-chain base. These are linked together by an amide bond. The fatty acid and the long-chain base may have different numbers of carbon atoms, double bonds, methyl branches and a varying number of hydroxyl groups (11,17).

A glycosphingolipid is an amphipathic molecule. That means that it has one water-soluble part, the carbohydrate chain, and another part with low water solubility, the ceramide. In a water environment the ceramide parts tend to avoid the water (compare olive oil in water forming droplets, 18) and mix with chemical moieties having similar properties, such as found in cell membranes.

Thus, according to most current views, the glycosphingolipids are anchored to the plasma membrane (or other membranes) by the ceramide, with the carbohydrate chain sticking out (11) as shown in Fig. 2.

Carbohydrate chain Lipid part (Ceramide)



GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer

Fig. 1. Schematic formula of the glycosphingolipid globotetraosylceramide (globoside). This glycolipid is known to be active as the blood group P antigen (10,140) and as a receptor for the binding of bacteria to a cell surface (paper V, see below).

The abbreviated designation below the formula is according to Refs. 12 and 13. The different sugar residues are shown as GalNAc for N-acetylgalactosamine, Gal for galactose and Glc for glucose. The type of bond is shown as α/β1→n indicating anomeric configuration (α or β) and binding position (n). In addition, to define the saccharide chain structure completely it would be necessary to indicate the absolute configuration (D or L) and ring size (p = pyranose = 6-membered and f = furanose = 5-membered ring) for each carbohydrate residue. Thus the complete designation should be D-GalNAcpβ1→3-D-Galpα1→4-D-Galpβ1→4-D-Glcpβ1- 1Cer (compare Table I and note (*) of Table VI of appendix).

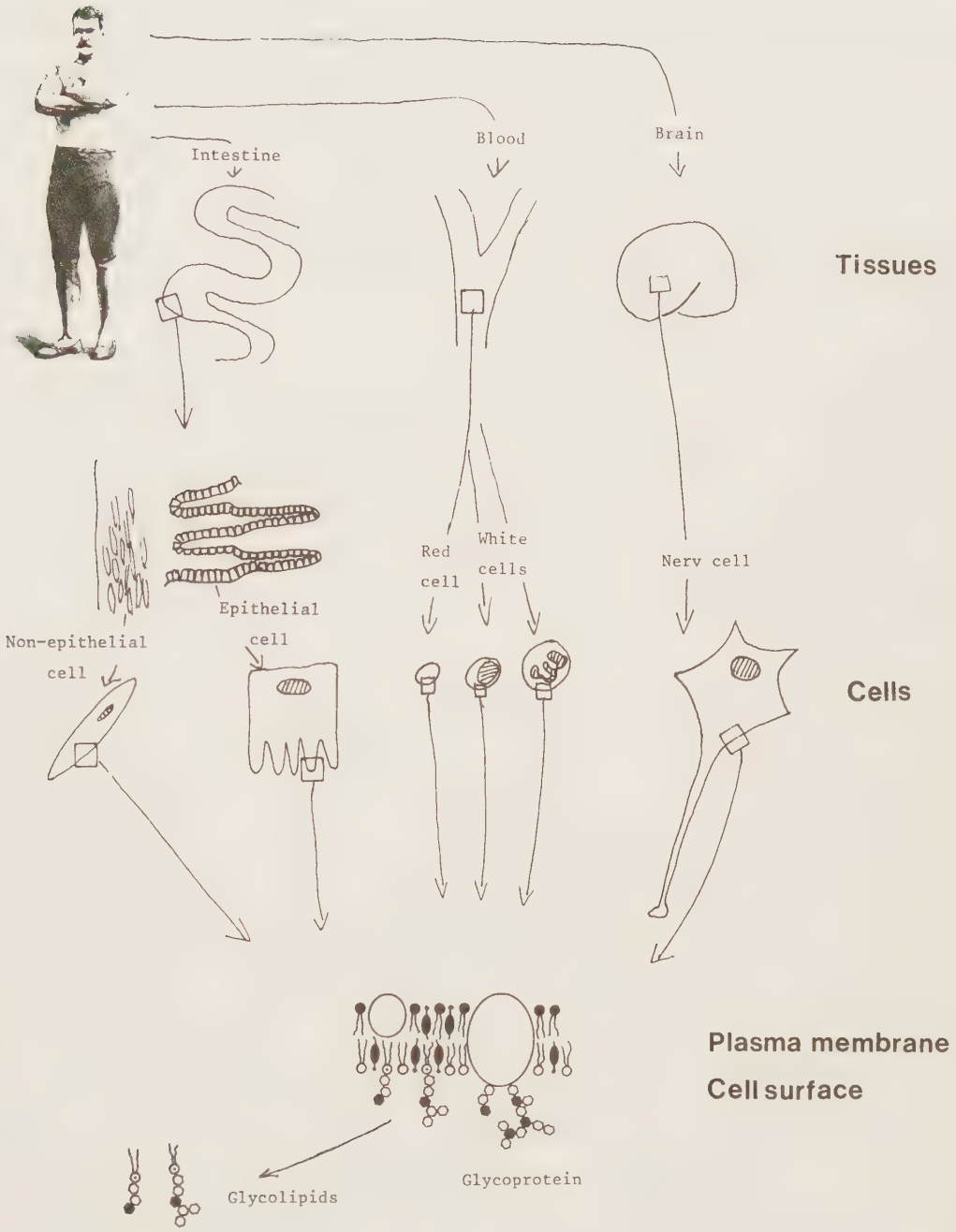


Fig. 2. Schematic illustration of the localization of glycosphingolipids in the body. Each tissue is built up of different cell types. Each cell is surrounded by a membrane separating the interior from the outside. The membrane is thought to be composed of a double layer of lipids containing "islands of proteins" (19,20). The glycosphingolipids are found in the outer part of the lipid bilayer with the carbohydrate chain sticking out at the cell surface (10,11,21). There the carbohydrate may be recognized by other cells or agents, compare Fig. 3.

From this it may be assumed that the glycolipids have at least two types of functional roles in the cell (10,11). Firstly, they may, primarily through the carbohydrate chain, serve as a cell surface marker or receptor and take part in cell surface recognition (Fig. 3 gives an example). Secondly, they constitute a component of the membrane and affect its physico-chemical properties primarily through the ceramide part.

A characteristic feature of glycosphingolipids is the distinct variations of structure between species and tissues as illustrated by the following remark on the difference between human and horse erythrocytes (23):

"It seemed almost inconceivable that the difference of species would bring such a distinct difference in the composition of lipid."

A review of glycosphingolipid structures and occurrence are found in the appendix, and species, tissue and individual diversity is discussed below. Also in the appendix, "cell surface recognition" is defined and the role of glycosphingolipids is exemplified and discussed.

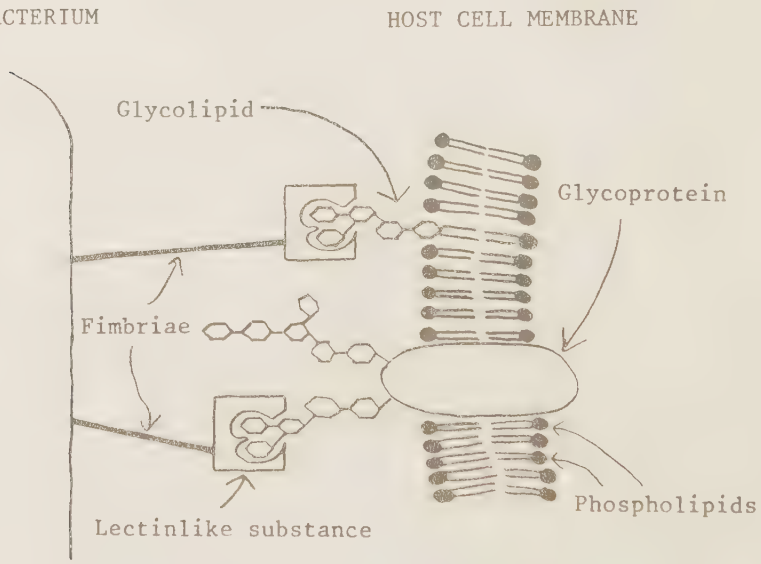


Fig. 3. An example of the involvement of glycosphingolipids in cell surface recognition. Model for the adherence of bacteria to cell surface. The saccharide chain of a certain glycolipid (or glycoprotein) is "recognized" by a lectin-like substance on the bacterial surface through mutual fitting of shape and chemical properties. (Analogous to lock and key). See appendix and paper V. This may be an important step resulting in infection of the host. From Ref. 22.

2. THE PRESENT STUDY: CONTEXT AND APPROACH

The present study is part of a larger project with the grant title "Structure and function of glycosphingolipids with special reference to surface structures of the cell". The main part of the experimental work was devoted to a static description of the glycosphingolipid composition of small intestine (papers I-IV). This should provide an essential part of the knowledge necessary for an understanding of the function of this tissue on a chemical level. A comparison of the glycolipids found in small intestine from different species and in different parts of small intestine was expected to give clues as to how specific glycolipid structures are related to tissue function. Small intestine was chosen for investigation because it was reported to be a rich source of glycosphingolipids (24,25), including blood group antigens. In addition, the rapidly renewing epithelial cells of small intestine may be isolated from non-epithelial tissue and separated according to maturity by a simple procedure (26). Therefore, the small intestine should provide a suitable experimental system for the study of glycosphingolipid changes during cell differentiation (27,28) and also for the study of tissue specificity of glycosphingolipid patterns (papers I and IV). In order to select the most suitable experimental animal for such studies the glycosphingolipid pattern of different animals was investigated (papers II,III, Ref. 29). A comparative study has also an interest of its own as it is a useful tool to increase the understanding of the relation between chemical structure and biological roles of compounds (30).

The rat was chosen as experimental animal for more detailed studies (27,28,31-41, paper IV). This was because rat small intestine showed the most complex glycosphingolipid pattern. Therefore, it was considered to provide the best chance that at least some of the glycolipids should show interesting changes during epithelial cell maturation.

A prerequisite for the studies was the availability of efficient methods for the purification (42, K.-A. Karlsson unpublished) and chemical analysis of glycosphingolipids (43-45). These methods were supplemented and developed further during the work (papers II-IV).

Finally, part of the experiments were intended to test the role of glycosphingolipids in a specific case of cell surface recognition, i.e. the adherence of Escherichia coli to human urinary tract epithelial cells in urinary tract infection (paper V). Blood group activities of intestinal glycolipids were also tested (papers I-III).

3. METHODOLOGY

The chemical characterization of the glycolipids of a tissue involves the following steps: extraction, fractionation (including separation of glycolipids from other components and from each other) and chemical analysis of mixtures of glycolipids or isolated glycolipids.

3.1. Extraction and fractionation

The methods used in the present work were designed to give at first: total fractions free of non-glycolipid contaminants, of non-acid glycosphingolipids (28, K.-A. Karlsson unpublished and papers I-III,V), gangliosides and sulphoglycosphingolipids (36, paper IV), respectively. After this the glycolipids were further separated from one another (used in paper IV) or the total fraction taken directly for chemical analysis (see below).

How total were the fractions? The glycosphingolipids pose a special problem in this respect, because of the wide range of solubilities for molecular species having different carbohydrate chain lengths (9,24). Thus, for example, the polyglycosylceramides possibly having up to 60 carbohydrate residues were not detected until more polar extracts were analysed (14-16). It may be assumed from literature data and experiments (K.-A. Karlsson unpublished) that larger glycolipids partially or completely remain in the insoluble tissue residue or on columns. Also other types of glycolipids, for example complex phosphoglycosphingolipids (found in plants (46) and evertebrates (47) but so far not in mammals) and polysialogangliosides (48, compare 49) could theoretically be partially or completely lost. Glycolipids may also possibly be lost if they are complexed to other compounds (e.g. proteins) in the tissue (50-52). It should also be noted that the purification procedures used include mild alkaline treatment. Therefore, naturally O-acetylated glycosphingolipids (8,53) and glycerolester-based glycolipids (9,54-56) are degraded.

3.2. Chemical analysis

Information on the chemical structure of glycosphingolipids is often initially obtained from the chromatographic behaviour of the component. Useful preliminary information of structure may also be obtained with specific staining reagents demonstrating for example carbohydrate (9,57), sialic acid (58) and non-glycolipid contaminants in the sample (42,57). For a more detailed chemical characterization other methods are used alone or in combination as outlined in Table I.

Table I. Methodology for the determination of different structural parameters of glycosphingolipids. The evaluation applies to the analysis of pure compounds if not otherwise stated. Mass spectrometry of intact glycolipids is described in Refs. 9,43,44,59,60, papers II-IV, the analysis of long-chain bases in 9,61 and the other methods in 9,24 and references below.

	Carbohydrate chain length	Carbohydrate composition	Carbohydrate sequence	Binding positions	Ring size	Anomeric configuration	Absolute configuration	Ceramide composition
Mass spectrometry of intact derivatized glycolipids	Exact (*)	Exact for carbohydrate (*) types (Hex, HexN, Fuc etc)		In special cases		-	-	Good (*)
Chromatography of intact native or derivatized compound	Rough estimate							
Degradation + identification/ quantification of the released saccharides and/or lipid parts by gas chromatography and/or other methods	Good estimate	Good estimate	-	-		(**)	(***)	Good estimate
Partial degradation (enzymatic or chemical) followed by analysis of the released saccharides (as above) and glycolipid product	Good estimate	Good estimate	Good estimate	In special cases (****)		(*****)	-	-
Degradation of permethylated or permethylated-reduced compound + analysis of the released saccharides by gas chromatography - mass spectrometry or other methods	-	-	Partial	Exact or partial		-	-	-
NMR spectroscopy	Exact	Partial in some cases				Exact	-	-
Optical rotation measurement	-	-	-	-		Estimate	-	-
Immunological analysis	-	Indicates the presence of certain immunological determinants						-

(*) Marks that the information may also be retrieved for and assigned to individual compounds in a mixture.
(**) By chromium trioxide oxidation (62).
(***) D or L form of released saccharides may be determined by gas chromatography of special derivatives (63).
(****) By periodate oxidation (Smith degradation).
(*****) By use of pure specific glycosidases.

In the present work, two different approaches to the chemical characterization of glycolipids have been used. In the first (papers II and III) glycolipid mixtures were analysed by thin-layer chromatography, mass spectrometry and immunological analysis. This was done in order to outline comparison of the structures present in many samples before the time-consuming purification of single components was started. In the second approach individual glycolipids were first purified, followed by a more complete chemical analysis. This was used for a detailed analysis of rat small intestine (paper IV).

3.2.1. Mass spectrometry. The method of mass spectrometry has a central position in the present work. The principles of the technique are outlined in Fig. 4. It is now applied to a wide range of problems in biochemistry and medicine (60). The Department of Medical Biochemistry in Göteborg has a tradition of mass spectrometry since the pioneering works of the late Professors Stina and Einar Stenhagen on the technical development of the equipment (coupling of gas chromatography - mass spectrometry) and on the application to analysis of fatty acids (60). The tradition has been continued by our group by the application of mass spectrometry in lipid and carbohydrate analysis (mainly long-chain bases (61) and glycosphingolipids (44)). This work has also been pioneering in the analysis of high-molecular-weight compounds (39,60,64). The latter field is currently subject to much interest since it is hoped that mass spectrometry may be used for analysis of intact proteins and other complex biomolecules. Although the largest fragment so far observed in a mass spectrometer had a mass above 9,000 (65), the analysis of compounds above 500 molecular weight is still rare (60). The successful analysis of complex glycosphingolipids did not depend primarily on novel technical equipment but rather on a careful selection of derivatives and conditions for sample handling and analysis (44). Thus, the use of acetylated or trimethylsilylated derivatives only allowed the identification of the sequence of 3 sugar residues. The permethylated and permethylated-reduced derivatives, used in the present work, allows the identification of the sequence of at least 12 sugar residues (39).

The usefulness of mass spectrometry of intact glycolipids compared to other methods is commented upon in Table I and in papers II and III. Mass spectrometry is unique in affording exact structural information that can be assigned to individual components in a mixture. A prerequisite for this is simple, informative and reproducible spectra as obtained for permethylated and permethylated-reduced derivatives.

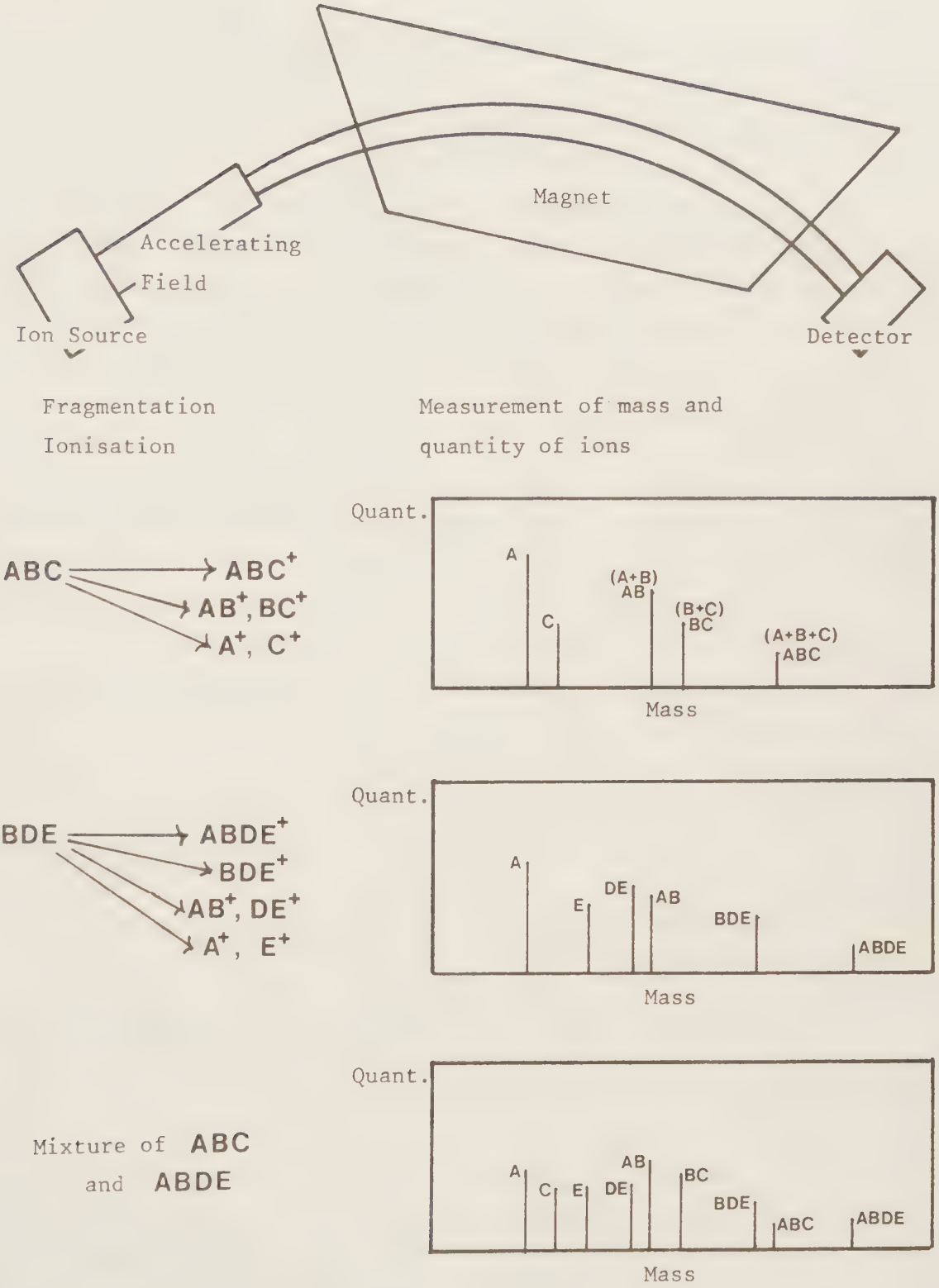


Fig. 4. Principles of mass spectrometry. Top: schematic picture of the instrument. Middle and bottom: the process taking place and the information retrieved.

The molecule is split into a mixture of charged fragments (including the charged intact molecule) in the ion source. The mass of each fragment is measured by acceleration in an electric field and deflecting in a magnetic field. The mass spectrum (right) shows the amount (vertical axis) of fragments with different mass (horizontal axis). The masses reflect the chemical constitution of each fragment and are fitted together (interpreted) to indicate what the original chemical structure was (e.g. ABC or ACB?).

4. SPECIES, INDIVIDUAL AND TISSUE DIVERSITY OF GLYCOSPHINGOLIPID STRUCTURES

4.1. Description of glycolipid diversity

The present results (papers I-IV) extend the finding of Suzuki et al. (25) and McKibbin et al. (24) that small intestine is rich in glycosphingolipids for several species. They also show that the pattern of glycosphingolipids varies considerably for different species and also for individuals of the same species, and that many species have large amounts of blood group type glycolipids in their small intestines.

It was also shown that the glycolipid pattern of epithelial cells is different from that of non-epithelial tissue of human (paper I) and rat small intestine (paper IV, Fig. 5) as indicated previously for some glycolipids of dog by immunofluorescence (66). This was also indicated for other species by the present results on ceramide composition of different glycolipids (Table II in paper III).

The study presented in paper IV showed that even closely related cells may have different glycolipid composition, i.e. epithelial cells of duodenum versus jejunum-ileum (Fig. 5). Finally, the chemical part of paper V showed that also epithelial cells of urinary tract (sediment) may contain a high concentration of blood group type glycolipids (Le^a active pentaglycosylceramide).

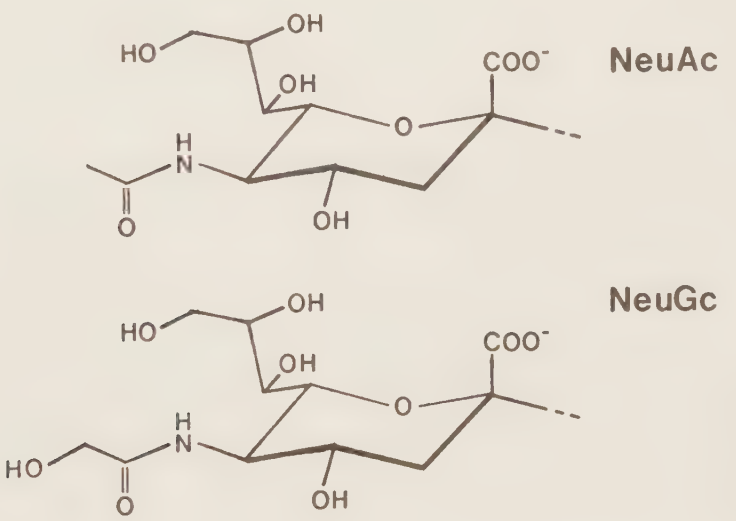
The results contribute in an interesting way to the general picture of glycolipid diversity as described below.

4.1.1. Which glycosphingolipids and parts of glycosphingolipids are involved in the variations? Both the carbohydrate chain structure and ceramide structure vary between different species and tissues and the carbohydrate chain also between individuals.

All glycosphingolipid types are involved in the variations. Thus, the present studies and literature data show that, for small intestine, the pattern of fucolipids and other neutral glycolipids including cerebrosides (24,25,27) vary between species (papers I-III). They also show that the presence of hematosides (papers I,IV) and other gangliosides (36,67) and also sulphatides (compare paper I with Ref. 27 and Fig. 5 in paper IV) vary between species. No single glycolipid except, possibly, glucosylceramide was present in all species, in amounts within the same order of magnitude (minor quantities cannot be excluded).

If the results for small intestine mucosa are compared with data on other tissues, it seems as if variations between tissues also involve all types of glycolipids (9,10,27,48, paper I). The present results illustrate this for hematosides of small intestine of the rat, Fig. 5 (paper IV). Thus, the sialic acid of the hematoside was different for epithelial cells as compared to non-epithelial tissue of jejunum-ileum. It was also different for epithelial cells of jejunum-ileum as compared to duodenum.

Individual variations (Table II) include fucolipids in small intestine (paper III), erythrocytes and other cells, other neutral glycolipids in erythrocytes and other cells and hematosides and other gangliosides in erythrocytes. The presence of cerebrosides and sulphate-containing glycolipids has so far not been shown to vary between healthy individuals. However, an interesting difference in the amount of sulphatide and digalactosylceramide between male and female mice was reported (174,175).



	Duodenum	Jejunum-Ileum
Epithelial cells	NeuAc	NeuGc
Non-Epithelial tissue	NeuAc	NeuAc

Fig. 5. Structure of sialic acid residues in hematosides of various parts of rat small intestine (paper IV). NeuAc is N-acetylneuraminosyl- and NeuGc is N-glycoloylneuraminosyl- residues, respectively.

Table II. Individual variation of glycosphingolipids. Examples of structures present in some individuals but not others of the same species. Numbers and letters in square brackets below structure refer to Table VI in Appendix.

Structure	Comment
[1-5] Galα1→3Gal GalNAcα1→3Gal Fucα1→2Gal Fucα1→4Gal	Human blood group ABH, Lewis systems. Involve major glycolipids of small intestine (24,68) and meconium (69), minor components of erythrocytes (10,70). Blood group A substances (GalNAcα1→3Gal) of rat small intestine (38).
[B,8,10] Galα1→4Gal GalNAcβ1→3Gal	Human P blood group system. Involves major components of erythrocytes and other tissues (71-73).
[G] GlcNAcβ1→6Gal	Human Ii blood group system (74).
Gal1→4Gal GalNAc1→3Gal	Mouse kidney (anomeric configurations were not determined) (75).
[14] NeuGc	Dog erythrocytes have NeuGc or NeuAc in major component, hematoside (76).
[13,14]	Ox erythrocytes. Different relative amount of hematosides and major gangliosides of lactoneo series. Total gangliosides lacking in some individuals (77).
[20] 4-O-AcNeuGc	Horse erythrocytes. O-Acetyl group lacking in hematoside of some individuals (77).
[13] NeuAcGalCer	Mouse erythrocytes. Also other gangliosides vary (78).
[18] NeuGc	Cat erythrocytes. NeuGcNeuGc in most cats. NeuAcNeuAc in some (siamese) (79).

4.1.2. Some characteristics and regularities of glycolipid carbohydrate chain diversity. Extensive, often qualitative variations of glycosphingolipid patterns were found for small intestine and also other tissues such as erythrocytes (10) or thymus (80). On the other hand, the major glycolipids of brain seem to be identical for all mammals studied (48,80) (Table III). They are also the same in birds and related (belonging to the ganglio seres, Table VI) in fishes (48).

The variations for one tissue do not seem to structurally correlate with the variations for another tissue. Thus, the pattern of major glycolipids of erythrocytes of mouse (78), cat (86), guinea-pig (83) and rat (unpublished) is distinctly different to that of glycolipids of small intestine (paper III). Concerning man (paper I, 10) and rabbit (paper II, 81,83) on the other hand, there appears to be some structural relation between glycolipids of the two origins.

Thus, the species and tissue variation of extraneural glycolipids seems at first glance highly irregular. However, some interesting regularities may be found. Thus, all mammalian glycosphingolipids identified so far are built up of the sugar residues glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid and fucose. Mannose has not yet been found in any mammalian glycolipid despite the fact that this sugar is present in glycoproteins (87) and that the activated precursor (GDP Man) of this sugar (168) is probably present in most cell types. Glucose has been found linked only to ceramide and never in the outer part of the saccharide chain and fucose has not been found as an internal residue of

Table III. Species and tissue variations of glycosphingolipids. Example of the occurrence as a major component* of some glycosphingolipid types** in tissues of different species. One glycolipid type can have a different tissue distribution in different animals.

The data are based on the following references: for brain of Man, Rat, Mouse (48), Guinea-pig (probale,reference not found), Dog and Rabbit (80); for erythrocytes of Man (10), Guinea-pig (82), Dog (83) and Rabbit (81,83); for leukocytes of Man (84); for small intestine of Man (24, paper I), Rat (27, 104, paper IV), Mouse (85, paper III), Dog (24) and Rabbit (92, paper II,tentative).

	Brain	Erythrocytes	Leukocytes	Small intestine epithelium
Man	D	B	F	E
Rat	D	n.d.	n.d.	I B
Mouse	D	n.d.	n.d.	D
Guinea-pig	D	D	n.d.	n.d.
Dog	D	I	n.d.	F
Rabbit	D	F	n.d.	F

(*) More than 25 % of glycolipids containing two or more sugars.

(**) B, D, E, F same as in Table VI of Appendix, i.e.
B: globo series, contains Galα1→4Gal
D: ganglio series, contains GalNAcβ1→4Gal
E: lacto series, contains Galβ1→3GlcNAc
F: lactoneo series, contains Galβ1→4GlcNAc
I: hematosides, NeuAc- or NeuGc-GalGlcCer
n.d. not determined

mammalian glycolipids. Most mammalian glycolipids have one of the core structures or terminal parts listed in Tables II, III and VI (Appendix). In contrast to these, glycolipids of evertebrates and plants may contain carbohydrate chains that are very different to the mammalian types as exemplified in Table VII of the appendix.

Although the glycolipid pattern of a tissue varies between different mammals many glycolipid structures often reappear in several species. For example, blood group A determinant was present in small intestine glycolipids of cat, rabbit, rat, dog and man (paper III). These similarities of animals that do exist do not correlate with the degree of evolutionary relation of the species (Table IV). On the other hand, disialyllactosylceramide was found as the major glycolipid of erythrocytes in several species of Felidae (89) (i.e. cat, lion, hyena).

It is also an interesting fact that a glycolipid which is present in a certain tissue of one animal but is lacking in this tissue of a second animal may be present in another tissue of the latter. Some examples are given in Table III. Thus, the glycolipids belonging to the lactoneo series are major glycolipids of epithelial cells of dog small intestine (24) and possibly cat and rabbit small intestine (papers II,III, 92). In man such glycolipids are lacking in small intestine epithelial cells (paper I) but are present in non-epithelial tissues (9,10) and are probably major components in white blood cells (84). Another example is the ganglio series of glycolipids. Members of these are the gangliosides found in the brain of all species but in the mouse another member, gangliotetraosylceramide, is also found as the major component of small intestine epithelial cells (paper III, 85) and possibly in other mouse tissues (121,122). The same phenomenon is observed for the globo series of glycolipids, hematosides (Table III) and many other types of glycolipids. Thus, species variations among mammals is to an important degree based on different tissue distributions of a limited number of glycolipid types and only partially on different glycolipids in each animal as a whole. Indeed, as the glycosphingolipid composition of the whole body is not known for any mammalian species and many tissues have not been studied at all, it may theoretically turn out that every glycolipid type will be found in some tissue or stage of development for each species.

4.1.3. Variation of ceramide structure. Epithelial cell glycolipids (of small intestine and others) seem in general to contain more

Table IV. Taxonomic relation of some species (90). The presence of blood group A and B antigen and Forssman antigen in small intestine (see structures 1, 2 and 9, respectively, in Table VI b of Appendix) identified chemically and immunologically as in papers II,III and Refs. 9,24,27,33,40,66,85,91. Presence is marked as E if localized to epithelial cells and as NE for non-epithelial tissue. The localization for cat, rabbit, mouse, guinea-pig and hen was concluded from the ceramide composition (see paper III).

Class	Order	Family	Genus	Species, Individuals		A-antigen	B-antigen	Forssman antigen
Mammalia	Primates	Hominidae	Homo	Man	-	E	-	-
					-	-	E	-
					-	-	-	-
	Carnivora	Canidae	Canis	Domestic dog	-	E	-	NE
					-	-	-	NE
		Felidae	Felis	Domestic cat	-	E	-	NE
	Rodentia	Muridae	Mus	House mouse	-	-	-	NE
			Rattus	Norway rat	-	E	NE	-
					-	-	NE	-
		Cavidae	Cavia	Domestic Guinea-pig	-	-	-	NE
	Lagomorpha	Leporidae	Oryctolagus	Domestic rabbit	-	E	E	-
Aves	Galliformes	Phasanidae	Gallus	Hen	-	-	-	NE

hydroxylated ceramide types compared to those of non-epithelial cells (erythrocytes and others) (11, papers I-IV). The ceramide structure of glycolipids of one tissue may also vary between species. For example, in the rat epithelial cells ceramides contain only trihydroxy base combined with non-hydroxy fatty acids and hydroxy fatty acids (27, paper IV) while in man they contain dihydroxy or trihydroxy base combined with almost exclusively hydroxy fatty acids (93).

4.2. Some functional and technical implications of glycosphingolipid diversity

The map of glycosphingolipid compositions is at present very fragmentary even for the best studied mammals. However, the data available allow some points to be discussed.

4.2.1. The "heterophil" character of glycolipid diversity. Two of the characteristics of mammalian glycosphingolipid diversity may be summarized as follows: firstly, the distribution of one specific glycolipid structure in one tissue of several species does not correlate with the evolutionary relationship of the species. Secondly, the tissue distribution of one glycolipid type may be very different for different species (compare Table III).

Such characteristics are not found in general for biological molecules of biological activities most of which (as for example hemoglobins) are similar for closely related species and appear in the same tissue type (e.g. erythrocytes) for all animals. However, these characteristics were early defined for so-called heterophil antigens (see below). Therefore the adjective "heterophil" will be used below to draw attention to this type of diversity for both molecular structure and biological activities in general, e.g. heterophil species distribution and heterophil tissue distribution, respectively.

This could be useful on the one hand to indicate a relation between molecular structure and biological activity. On the other hand it could give clues to the underlying mechanisms for heterophil species and tissue distributions *per se*, possibly of general biological importance.

4.2.2. The evolution and expression of glycosphingolipid patterns. A survey of some known determinants of the glycosphingolipid pattern for a tissue is found in the appendix. The discussion below is for simplicity

restricted to glycosyltransferases. The arguments are not changed in principle if other factors are considered.

Evolution has been studied at the molecular level for many proteins (94), for example hemoglobins (95). The classical picture is that of successive changes of protein structure through changes of the coding DNA sequence combined with gene duplications to give rise to families of related proteins. Although additional mechanisms may be revealed by the recent direct studies of genome organisation (96) the result of this type of evolution is: the amino acid sequence of proteins correlate with the degree of evolutionary relation of species. It is reasonable to think that a similar type of evolution of glycosyltransferases (suggested in ref. 97) may explain the large differences of glycolipid structures in mammals as compared to invertebrates (Tables VI and VII), i.e. changes over a long time range.

May it also explain the heterophil variations of glycolipid structures within the group of mammals or are additional mechanisms involved? In the first case (no additional mechanisms) the amino acid sequence of glycosyltransferases would not show the heterophil variations but the specificities would reappear accidently in unrelated species and in different tissues. This seems however unlikely. Alternatively, it is possible that the genes for all types (defined by specificity) of mammalian glycosyltransferases are found in each species. Thus, the heterophil species variation would not be found for the presence or absence of glycosyltransferase genes (compare discussion above for glycolipids of whole animals). However, then the varying expression of the genes in different tissues for different species requires an explanation.

Therefore, additional mechanisms are probably involved in the evolution of mammalian glycolipid patterns. One may only speculate on what they are. For example, the heterophil tissue variations may be due to a change in position of glycosyltransferase genes (going from one species to another) relative to genes for specific tissue functions (compare with the case for transposable elements (96) and endogenous virus genomes (98)). The expression of some glycosyltransferases could become linked to (accidently or purposefully, see below under glycolipid functions) the expression of the latter "functional" genes.

A closer relation of endogenous virus DNA from cat and monkey as compared to other DNA of these species (i.e. a heterophil species variation

of DNA sequences) has been taken as evidence for "the horizontal transmission and subsequent perpetuation of the viral genes through the germ line" (99). Does this happen for glycosyltransferase genes?

4.2.3. Glycosphingolipid function. Several questions may be raised on how glycolipid structural diversity is related to glycolipid function:

Does glycolipid structure correlate with tissue function? One such case may concern the role of the ceramide as a building part of the membrane. In line with this the study of papers I,IV and others (11) indicated that the ceramides of epithelial cell glycolipids are more hydroxylated than the ceramides of non-epithelial glycolipids in several species. This has been suggested to be an adaptation to the different environments of the epithelial cell (see above) in order to stabilize the membrane (11,100). Also the carbohydrate chains of glycolipids may affect membrane stability (compare Ref. 101). Accordingly, the epithelial cells of all species contained a high concentration of glycosphingolipids. A second example is the close correlation between the amount of galactosylceramide sulphate (sulphatide) and Na^+-K^+-ATPase activity in several tissues of various species (11,102, 103). Both cases indicate an evolutionary conserved structure-function relationship. This may also apply to the major glycosphingolipids of brain which have an identical structure for all mammals (48).

On the other hand, no structure-function relation is apparent for many glycolipid carbohydrate chains. For example, N-glycoloylhematoside was found to be a major glycolipid of the epithelial cells of rat jejunum and ileum (paper IV, 104). It was not present in duodenum and not in non-epithelial tissue and its concentration increased 5-fold during the change of epithelial cells from immature cells in the crypt to mature villus tip cells. All this indicates that the organism spends energy on synthesis and regulation of this glycolipid. However, N-glycoloylhematoside is absent from the jejunum-ileum epithelial cells of other species such as mouse and man. Another case of missing structure-function relation is the presence of globotetraosylceramide as the major glycolipid in erythrocytes of most humans but the lack of it in some healthy individuals (of blood group p, 71,72). Are the corresponding functions taken over by some other molecules in the latter species or individuals requiring another carbohydrate chain structure? This could be possible if the glycolipids fulfil a function through the interaction with a specific ligand. A number of carbohydrate

binding proteins have been found in animal tissues. However, according to the limited data available they do not seem to vary much in specificity between species (105). It is therefore an interesting question if, in animal tissues, carbohydrate binding proteins exist for which the specificity has a heterophil species and tissue distribution, and even individual variation parallelling the glycolipid structures. Alternatively, the function itself, in which a glycolipid is involved, may vary between species and individuals (compare for example the case of vitamin C synthesis, 106).

It has been pointed out that "economy has not been a major consideration in the process of evolution" (107). If this is true also for substance classes other than DNA, glycolipid carbohydrate chains may be expressed (for unknown reasons, see above) without having any function at all. In special cases a certain glycolipid may have become involved in a specific process and then a conserved structure-function relation might appear, as exemplified above. However, despite a lack of function within an individual, glycolipids may be biologically active in self-not-self interaction (as antigens and in host-pathogen interactions, see below).

4.2.4. Glycosphingolipids in embryogenesis. In the present study Forssman and globotetraosylceramides were found localized to non-epithelial tissue of small intestine in several species and not to the epithelial cells (paper III). It was considered that this might reflect the embryonic origin (mesenchymal) of the non-epithelial tissue. Accordingly, in immunofluorescence studies antibodies against these glycolipids only labelled mesenchymal tissue (66,108,109). However, the picture is not simple as distribution among mesenchymal tissues was not the same for all species (109). For example, Forssman antigen is present in erythrocytes of some species (sheep, goat) but in another it is instead in the kidney (guinea-pig).

Immunofluorescence studies using antibodies against carbohydrate antigens have also been done on embryonal and differentiating tissues (110). Recently, several studies on early mouse embryos were reported (111-114). They may be summarized as follows: early mouse embryos are labelled by antibodies against Forssman antigen, blood group I, i and H antigen and against Fucα1$\rightarrow$3GlcNAc containing structures. For each antigen some cells are labelled and others not. The antigen may appear and disappear during development (i.e. act as a differentiation antigen). No certain relation to cell lineage was found for the Forssman antigen (115). It was suggested

that the consecutive expression and action of glycosyltransferases may cause the appearance and disappearance of a certain antigenic specificity (114).

In the present context it is interesting to ask if the expression of glycolipids and related carbohydrate structures in early embryos of other species, e.g. rat, are similar to the mouse or show a heterophil species and tissue distribution. In the first case this would indicate a conserved structure-function relation and lend further support to the suggestion that glycolipids play an important role during embryogenesis. However, in the second case the special considerations discussed above under glycosphingolipid function must be taken into account.

4.2.5. Glycosphingolipids in tumours. The glycosphingolipid pattern of transformed or tumour cells may be changed in a number of different ways compared to normal cells (110). Considering the heterophil variations of normal tissue glycosphingolipid patterns taken together with often large genome rearrangements of tumour cells (e.g. changed caryotype) this is not surprising. Regardless of whether the glycolipid changes are a decisive step in the transformation or not they are interesting. Firstly, new tumour specific antigens may be produced (110,116). Secondly, the expression of glycolipids may signal the expression of other genes giving clues to the mechanisms for tumour development.

4.2.6. Glycosphingolipids as heterophil antigens. The classical heterophil antigen, the Forssman antigen, was characterized as follows (117): it is present in sheep erythrocytes but antibodies against it can be produced by injecting tissues of guinea-pig and some other species into rabbits. The species in which it can be demonstrated are distributed all over the animal kingdom, bacteria and plants without any apparent correlation with evolutionary relations (117,118). This and other similar reactions (see 118) seemed at the time to violate the roles of serological specificity saying that when a tissue is injected into an animal antibodies should be produced against the tissue of the same or closely related species. When concepts of chemical substances and molecules were taken into account, the heterophil phenomena could be explained as immunological reactions against substances (or parts of substances) present in several different species. This has now been shown to be true as the Forssman antigen has been identified as a certain glycosphingolipid (Table VI) in several species (9). The serum sickness heterophil antigen has been identified as N-glycoloylneuraminic acid presented as part of different

glycolipids (10,110). The characteristics of the species variation of glycolipid patterns as described above make it probable that almost all glycosphingolipids may act as heterophil antigens.

In many experimental systems immunological reactions between species (and individuals) are used, for example heterologous immunizations to produce antibodies or study of the reaction of patient sera with antigens of other species (or individuals). In all cases when whole tissues or partially purified antigens are used for immunization or assay of sera the presence of heterophil antigens (and blood group antigens) must be considered and these may have irregular distributions among tissues, species and individuals as described above for glycosphingolipids. For example, during studies of anti-rat colon antibodies in sera of patients with ulcerative cholitis antibodies against human blood group A antigens were also found (119,120). Gangliotetraosylceramide has recently been suggested as a specific marker for natural killer (NK) cells in the mouse (110,121, 122), T lymphocytes in the rat (123) and human lymphoblastic leukemia cells (124), and an auto-immune antigen of lupus erythematosus dissematosus (125). However, this glycolipid is also a major component of mouse small intestine epithelial cells (paper III, 85). The antigens expressed in embryogenesis discussed above also act as heterophil antigens.

4.2.7. Species variability of other complex carbohydrates. It is an interesting question whether other saccharide chains will be found to vary between species as extensively as the glycolipids. For example, human milk contains complex free oligosaccharides (126), many of which resemble the saccharide chain of some glycosphingolipids including those of small intestine (24, paper I) and meconium (127). How does the pattern of saccharides in milk vary between species? The major saccharide of milk for all mammals studied is lactose. Some variations have been observed for more complex saccharides. However, in distantly related species (egg-laying mammals) the major saccharide was 4-O-acetylsialyllactose (128) and difucosyllactose (129) instead of lactose.

4.2.8. Different species as sources of antigens and receptor molecules. Carbohydrate antigens and receptor molecules are often difficult to prepare in sufficient quantities for experimental studies and they are difficult to synthesize. The heterophil characteristics of glycolipid diversity make it possible that at least some tissues of some animal can provide a rich source for a certain glycolipid. Therefore, a survey of glycolipid compositions is of direct practical importance.

5. THE ROLE OF HOST GLYCOLIPIDS IN AN INFECTIOUS PROCESS

5.1. Introduction

Bacteria in nature tend to grow attached to surfaces (130). A number of data indicate that the binding of bacteria to epithelial cells is an important step in the establishment of infection in animals and man (3,131, 132). One reason for this may be that unattached bacteria are washed away by secretions, for example urine in urinary tract infection. Another reason may be that a close proximity between bacteria and host cell surface is necessary, for example for the action of bacterial toxins.

The ability of bacteria to associate with mammalian cells varies between cells from different species, tissues and even individuals (3,131,133, 134). Each bacterial strain has a special range of cells to which it can bind. Therefore, the specificity of the interaction between bacteria and host cells may determine which host and which tissue becomes infected by a certain strain (134,135). For example, *E. coli* strains isolated from patients with urinary tract infection have a greater tendency to attach to human urinary tract epithelial cells than other *E. coli* strains (136). The species and tissue diversity of glycosphingolipid patterns described above make these compounds candidates for the role as host determinants of species and tissue specific bacterial attachment. This possibility was investigated for the case of attachment of pyelonephritic *E. coli* to urinary tract epithelial cells by several types of experiments (paper V).

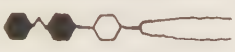
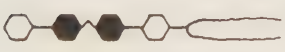
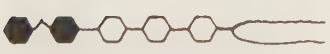
5.2. Present work

Taken together, the results indicated that *E. coli* strain 3669 interacts with uroepithelial cells and erythrocytes by a ligand-receptor type of interaction (Fig. 3, see Introduction and Appendix) and that the receptors are a group of structurally related glycosphingolipids (see Fig. 2 of paper V).

To define exactly the chemical specificity of the interaction purified ligands and receptor variants must be studied as has been done for antibodies and lectins (137,138). The present study was a step towards this on the receptor side. Although minor highly active impurities in the glycolipid fractions cannot be ruled out *E. coli* 3669 seems most probably to interact with both globotriaosylceramide and globotetraosylceramide. One explanation of this is that the bacteria carried ligands binding to the Galα1$\rightarrow$4Gal part found as a characteristic part in these glycolipids. The reason that globo-

tetraosylceramide is a more efficient inhibitor of attachment could be that the terminal galactosamine contributes to the site bound to the bacterial ligand or other factors such as solubility (see paper V). Whether other glycolipids also containing this part (Table VI) are recognized by the bacteria remains to be tested. Another explanation would be the presence of several related ligands on the bacteria, recognizing different glycolipids. The possibility that the amount and specificity of these ligands may vary between globoside-recognizing E. coli strains and with growth conditions must also be taken into account. For example, among a larger group of strains which all recognized globotetraosylceramide coated onto erythrocytes only about half also agglutinated sheep erythrocytes. In addition, other factors may contribute (see Ref. 3), such as the modification of receptors by bacterial enzymes.

Globotetraosylceramide and globotriaosylceramide are also the P and P^k antigens, respectively, of the human P blood group system (139) (Table VI, Fig. 6). Consequently, the glycolipid fraction from P_1, P_2 but not from p erythrocytes, lacking these glycolipids (71,72) inhibited adhesion of E. coli 3669. Källenius et al. (142) found that E. coli strains from pyelonephritis patients agglutinated P_1, P_2 and P_1^k but not p erythrocytes (as confirmed for 3669 and other strains). Based on this and also since the oligosaccharide of globotriaosylceramide inhibited the haemagglutination they suggested that globotriaosylceramide was a receptor for the

Antigens		Phenotypes				
Schematic structure	Name	P_1	P_2	P_1^k	P_2^k	p
		75%	25%	rare	rare	rare
	P^k	+	+	+	+	-
	P	+	+	-	-	-
	P_1	+	-	+	-	-

= Galα1→4Gal

Fig. 6. The P blood group system (139-141).

bacteria. The minimal receptor recognized was proposed to be Galα1→4Gal since a synthetic derivative of this disaccharide also inhibited haemagglutination (143). Thus, the results of Källenius et al. and the results of paper V agree. However, according to the initial results of Källenius et al. the tetrasaccharide released from globotetraosylceramide (and $NaBH_4$ reduced) was not inhibiting haemagglutination. This is in contrast to what might be expected from the present results.

5.3. Further studies

Some further studies on the role of glycolipids for attaching E. coli in urinary tract infection may be summarized as follows: for studies of large numbers of bacterial strains we searched for a simple system to investigate if a bacterial strain can recognize globoside or not. For this we used guinea-pig erythrocytes or other animal erythrocytes coated with a globoside fraction from erythrocytes as in paper V (144). Mannose resistant agglutination after, but not before glycolipid coating was taken to indicate globoside recognition. The effect is specific in two ways. First, unrelated glycolipids (GM_1 ganglioside, sialyllactoneotetraosylceramide) give no effect or only a slight effect as for lactoneotetraosylceramide. Second, globoside recognition may be demonstrated for some bacterial strains and not others.

The property to recognize globotetraosylceramide was common among pyelonephritic E. coli. It may be demonstrated in combination with recognition of mannose residues (i.e. mannose-sensitive agglutination of guinea-pig erythrocytes, see Duguid et al. in Ref. 3) for some strains or alone for others (144,145). On the host side a genetic basis for susceptibility to infection was indicated by the fact that the blood group P_2 was less frequent (1/28) among patients with recurrent urinary tract infection compared to the normal population (25 %) (146). As blood group P_2 individuals have less of glycolipids containing the residue Galα1→4Gal (as measured in erythrocytes (147)) this indicates a role for such glycolipids in the infectious process. The effect may be due to a different density of receptors for attaching bacteria in cells of the urinary tract. Other mechanisms are also possible. For example, similarities of host glycolipid antigens and micro-organism antigen may affect the immunological defense of the host (compare Ref. 148).

5.4. Practical implications

If adhesion of bacteria is a decisive step in an infectious process, the identification of the chemical structure of the receptor has many obvious therapeutic implications. Thus, analogues of the receptor can be given as medicine to compete with the true receptor for the bacterial ligand and thereby prevent adhesion and hopefully also the infection (as tested for cholera toxin, 150). The potential usefulness of bacterial ligands (fimbriae) as vaccines has been demonstrated in animal systems (pigs and calves) and it is currently investigated in human disease (diarrhoea) (149). However, the fimbriae of two bacterial strains may be immunologically different despite their recognizing the same (or similar) receptor (145). A receptor analogue can be used to purify the ligand or the common binding part of the ligand for the use of vaccine or experimental study (as also done for cholera toxin, 151). A carrier-linked receptor analogue can also be used as a diagnostic tool, to investigate if a clinically isolated bacterial strain can bind to this receptor or not as done in the present work with glycolipid-coated erythrocytes (paper V). Glycolipid-based assays have also been used for bacterial toxins (152).

In many instances human infectious agents are studied in animal models. If glycolipids are important in such a case the species variability of glycolipid structures must be considered. Either an animal with similar glycolipid composition to man (at least in the tissue of interest) should be used. Or the host-pathogen interaction in the animal must be considered a separate (but hopefully analogous) system, possibly requiring identification of its own glycolipid receptor and use of another pathogen strain.

6. APPENDIX: INTRODUCTORY SURVEY OF GLYCOSPHINGOLIPID STRUCTURE AND OCCURRENCE AND THEIR ROLE IN CELL SURFACE RECOGNITION

6.1. Glycosphingolipid structures

Table V shows a commonly used classification of glycosphingolipids based on the presence or absence of ionic groups. Tables VI a and b give a survey of mammalian glycosphingolipid structures and part a shows a commonly used classification of core structures. Table VII gives an example of glycosphingolipid structures found in an invertebrate species.

Saccharide chains linked to other lipid moieties are also found. For example, glycolipids containing glycerol esters or ethers as hydrophobic part are found as membrane components, mostly minor, of a wide variety of animal species including mammals (9,54), and as major components in bacteria and plants (55,56). These glycolipids are not further discussed in this thesis. Therefore, the term glycolipid as used in the text generally refers to glycosphingolipids.

Table V. Types of glycosphingolipids.

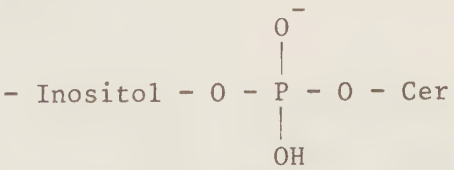
Type		Comment
Neutral		Contain only neutral sugar residues Gal, Glc, GlcNAc, GalNAc, Fuc, etc.
Acidic	Sialoglycosphingolipids (Gangliosides)	Contain also sialic acid residues NeuAc, NeuGc (compare Table II b)
	Sulphoglycosphingolipids	Contain also a sulphate group O_3SO^- (compare Table II b)
	Phosphoglycosphingolipids	Contain a phosphate group, for example  (46), or as in Table VII

Table VI. Synopsis of mammalian glycosphingolipids. Based on structures given in Ref. 8 and in Refs. given in the Table.

(a) Examples of core structures (*)	Proposed semisystematic names (Ref. 12) and comments
A Simple members	
Glcβ1→1Cer	Glucosylceramide
x→Galβ1→1Cer	Galactosylceramide
Fucα1→1Cer	Fucosylceramide
y→Galβ1→4Glcβ1→1Cer or Lacβ1→1Cer	Lactosylceramide
z→Galα1→4Galβ1→1Cer	Galabiosylceramide
B Globo series, contains Galα1→4Gal	
x→Galα1→4Lacβ1→1Cer	Globotriaosylceramide
y→GalNAcβ1→3Galα1→4Lacβ1→1Cer	Globotetraosylceramide
C Isoglobo series, contains Galα1→3Gal	
x→Galα1→3Lacβ1→1Cer	Isoglobotriaosylceramide
y→GalNAcβ1→3Galα1→3Lacβ1→1Cer	Isoglobotetraosylceramide
D Ganglio series, contains GalNAcβ1→4Gal	
GalNAcβ1→4Lacβ1→1Cer (w)	Gangliotriaosylceramide
x→Galβ1→3GalNAcβ1→4Lacβ1→1Cer (y)	Gangliotetraosylceramide
GalNAcβ1→4Galβ1→3GalNAcβ1→4Lacβ1→1Cer (z, z)	
E Lacto series, contains Galβ1→3GlcNAc	
GlcNAcβ1→3Lacβ1→1Cer	Lactotriaosylceramide
x→(Galβ1→3GlcNAcβ1)$_n$→3Lacβ1→1Cer (y)	Lactotetraosylceramide (n = 1) (n=2,combined with F,Ref. 127)
F Lactoneo series, contains Galβ1→4GlcNAc	
x→(Galβ1→4GlcNAcβ1)$_n$→3Lacβ1→1Cer (y)	Lactoneotetraosylceramide (n = 1) Blood group i antigens (n ≥ 2,Ref.198)
G Branched structures containing GlcNAcβ1→6Gal	
x→[GalGlcNAcβ1→6(3)GalGlcNAc]$_n$ Lacβ1→1Cer	"Megaloglycolipids" are proposed to be based on this structure (n ≥ 2,Refs.14-16)
may contain	
Galβ1→4GlcNAc and/or Galβ1→3GlcNAc (31)	Blood group I antigens (198)
H Others containing:	
x→Galβ1→3Gal	Muco series
x→Galβ1→4Gal	
x→Galβ1→6Gal	Branched structures
GlcNAcβ1→4GlcNAc	
y→(→3Galα1→)$_n$→Galα1→4Lacβ1→1Cer	(Refs.27,32,34,40)
z→Galβ1→3GalNAcβ1→3Lacβ1→1Cer	(Ref.199)

(*) The subdivision into core structures and terminal parts is arbitrary but follows in principle what is commonly used in the literature. x,y,z in part (a) indicates at which sugar residues terminal parts have been found (see below). The boxed sugars in part (b) belong to core structures. Therefore, some compounds are designated twice in the table e.g. the compounds under B in part (a) are the same as terminal part 8 linked to A,y and 10 linked to B,x respectively.

The structures are designated according to Refs. 12 and 13. The ring size and absolute configuration are not shown explicitly (compare Fig. 1). In all mammalian glycolipids for which they have been determined the pyranose form and the D configuration were found for Glc, Gal, GlcNAc, GalNAc and NeuAc and the pyranose form and L configuration for Fuc.

(b)	Terminal parts (*)	Found linked to (**)	Comment
	Neutral		
1.	GalNAcα1 → 3[Gal] (2 ↑ Fucα1)	A,y(27,33);E,x(24);F,x; G,x(27,33,39,198);H,x	Blood group A determinant (****)
2.	Galα1 → 3[Gal] (2 ↑ Fucα1)	A,y(185);D,x(27,40);E,x; F,x;H,z(199)	Blood group B determinants (****)
3.	[Gal] (2 ↑ Fucα1) Galβ1 → 3[GalNAc] (2 ↑ Fucα1)	A,y(35);B,y(127);C,x; D,x;E,x(91);F,x; G,x(31);H,y(40)	Blood group H determinant (***) (****)
4.	Galβ1 → 3GlcNAc (4 ↑ Fucα1)	E,y	Blood group Le^a determinant (****)
5.	Galβ1 → 3GlcNAc (2 ↑ Fucα1; 4 ↑ Fucα1)	E,x,y	Blood group Le^b determinant = a combination of H and Le^a determinants (****)
6.	Galβ1 → 4GlcNAc (3 ↑ Fucα1)	F,y	(****)
7.	Galα1 → 3[Gal]	A,y;B,x(34); F,x;G,x(200)	B antigens of rabbit erythrocytes(F,x;G,x)
8.	Galα1 → 4[Gal]	A,y; F,x	P^k antigen P_1 antigen
9.	GalNAcα1 → 3GalNAcβ1 → 3[Gal]	A,z;B,x	Forssman antigen
10.	GalNAcβ1 → 3[Gal]	B,x;C,x;H,y(27,32)	P antigen (B,x) Cytolipin R (C,x)
11.	Galβ1 → 3[Gal]	F,x	
12.	OAcyl → [Gal]	A,x(201)	
	Sialic acids		
13.	NeuAcα2 → 3[Gal]	A,x;A,y;D,w;D,x;D,v;D,x,y; D,z;F,x;G,x(110,210)	(****)

continued

(**) The designations refer to part (a) and show: group of core structures i.e. A B etc., position i.e. x y etc. (reference to literature).

(***) Fucose linked to lactotetraosylceramide (E, x) also proposed as Le^d determinant.

(****) In many instances two terminal parts are found linked to the same core structure. Structure 5 (Le^b antigen) is given as an example of this. Other examples are combinations of structures 1, 2 or 3 with 4 or 6 (45,70,91,139), structure 3 with 13 (210), 14 or 17 (211) and structure 6 with 13 (212).

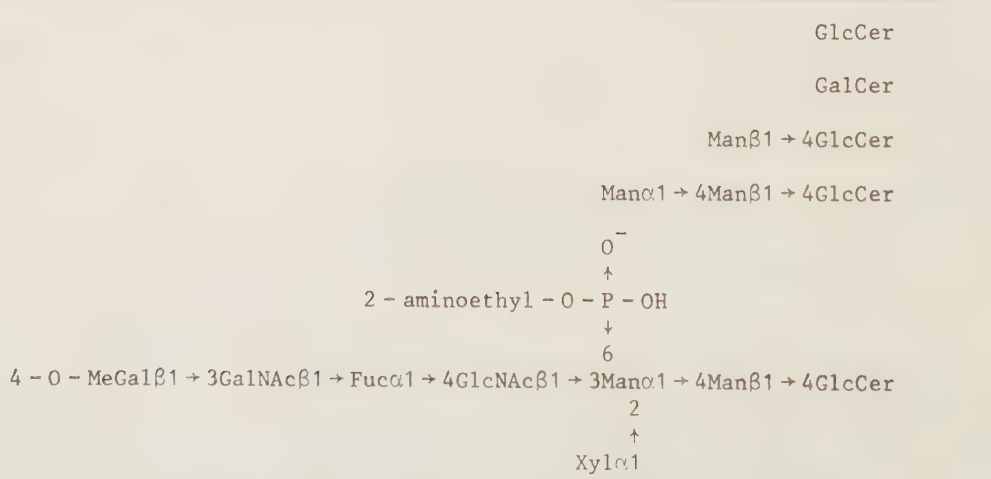
Table VI b, continued

(b) Terminal parts (*)	Found linked to (**)	Comment
14. NeuGcα2 → 3 $\boxed{\text{Gal}}$	A,y;D,y;D,x,y;F,x	(****)
15. NeuAcα2 → 6 $\boxed{\text{Gal}}$	F,x(202)	
16. NeuAcα2 → 3Gal $\boxed{\text{GalNAc}}$	B,y(203);C,y(36)	
17. $(\text{NeuAc}\alpha2-8)_n\,\text{NeuAc}\alpha2 \rightarrow 3\boxed{\text{Gal}}$	A,y(n=1);D,w(n=1);D,y; D,x,y(n=1-2);F,x(n=1,Ref.204)	(****)
18. NeuGcα2 → 8NeuGcα2 → 3 $\boxed{\text{Gal}}$	A,y	
19. NeuGcα2 → 8NeuAcα2 → 3 $\boxed{\text{Gal}}$	A,y(205)	
20. 4 - O - AcNeuGcα2 → 3 $\boxed{\text{Gal}}$	A,y	
21. 9 - O - AcNeuAcα2 → 8NeuAcα2 → 3 $\boxed{\text{Gal}}$	D,y(53)	(*****)
22. NeuAcα2 → 3GalNAcβ1 → 3 $\boxed{\text{Gal}}$	F,x(206)	
Sulphate groups		
23. $O_3SO \rightarrow 3 \rightarrow \overline{\text{Gal}}$	A,x;A,y;B,x(******); D,w(207)	
24. $O_3SO \rightarrow 6 \rightarrow \overline{\text{GlcNAc}}$	F,y(208)	
25. $O_3SO \rightarrow 8 \rightarrow \text{NeuAc}\alpha1 \rightarrow 8\text{NeuAc}\alpha1 \rightarrow 3\boxed{\text{Gal}}$	A,y(209)	

(*****) Combined with structure 13 D,x.

(******) Anomeric configuration not determined.

Table VII. Examples of invertebrate glycosphingolipids from the shellfish Corbicula sandai (47,158).


GlcCer
GalCer
Manβ1 → 4GlcCer
Manα1 → 4Manβ1 → 4GlcCer
O⁻
2 - aminoethyl - O - P - OH
6
4 - O - MeGalβ1 → 3GalNAcβ1 → Fucα1 → 4GlcNAcβ1 → 3Manα1 → 4Manβ1 → 4GlcCer
2
Xylα1

6.2. Where are glycosphingolipids found?

Various glycolipid types have different chemical properties and require different methods for extraction, purification and analysis. Therefore, available literature data do not only reflect the true distribution of glycolipids but also the methods available and the question asked in each investigation. Thus, the change of technology may reveal new types of compounds, such as polyglycosylceramides (14-16).

With these limitations some statements are still possible about the distribution of glycosphingolipids. For the most studied mammals, i.e. man, laboratory and domestic animals, glycosphingolipids have been found in all tissues studied. But the amount varies considerably. The brain is rich in gangliosides (9,48). Some extraneural tissues such as epithelial cells of gastrointestinal tract (24,25,153), bladder (154) and glandular organs (155) also contain large amounts of glycosphingolipids. Other tissues contain smaller amounts, e.g. red blood cells (9,10), liver (9), muscle (9). The amount within one tissue may vary for different species as exemplified by red blood cells (9).

For other sources the data are scattered, but glycosphingolipids have been found in a wide variety of invertebrates (for example 47,156-159), plants (46) and yeast (160), in some bacteria (161) but not others (162) and have been indicated in unicellular animals, for example slime mould (163).

Only a few studies have been done on the subcellular localization of glycosphingolipids. Subcellular fractionation showed the presence of glycolipids in brush borders of small intestine epithelial cells (92,213), plasma membrane and golgi apparatus of other cells (9). Immunofluorescence studies have indicated glycolipids on the cell surface (9,108) and also intracellularly (108). Labelling with galactoseoxidase has demonstrated globotetraosylceramide on the surface of erythrocytes (164,165) and its absence on the inner surface of the membrane (164).

6.3. What determines the glycosphingolipid pattern of a tissue?

The carbohydrate chain of glycosphingolipids is probably synthesized by the stepwise transfer of sugar residue from activated precursors catalyzed by different specific glycosyl transferases (88,166). The glycosyl transferases seem to be specific for the sugar transferred, the glycosidic bond formed (anomeric configuration and binding position) and the accepting saccharide (88,166-168). The process occurs in the Golgi apparatus and possibly also at other cell membranes. The saccharide chain may be further

modified (e.g. 168) by sulphatation, oxygenation (converts NeuAc to NeuGc), acetylation or methylation by other types of enzymes (compare Table VI for mammalian sialic acids). The breakdown of glycosphingolipids is catalyzed by different glycosidases probably in the lysosomes (169). The lack of a glycosyl transferase may give lack of a specific glycolipid in a cell (e.g. 170) and increased concentration of other glycolipids. The lack of a glycosidase may give accumulation of glycolipids in cells (169). However, the regulation of these processes resulting in a specific glycolipid pattern is unknown. Some knowledge of the genetic regulation of glycolipid expression comes from the studies on inheritance of blood group antigens (139). In most of these cases it seems as if each glycosyl transferase is inherited separately and the expression of a certain enzyme is dominant over the lack of it. The expression of a certain glycolipid depends on the interaction of several enzymes and therefore the inheritance may be more complicated. The glycolipid pattern may also vary with the physiological or pathological state of cells or whole animals (see Ref. 110, 171) and be modified by external agents such as butyrate added to fibroblast (172) or thrombin to thrombocytes (173) or hormones (174,175). Finally, it is important to note that most tissues are composed of many different cell types and that each cell type (papers I and IV) and possibly part of the cell may have different glycolipid composition. The pattern observed for a whole tissue is therefore the result of super-imposition of many glycosphingolipid patterns.

6.4. Cell surface recognition

Cells interact with the surroundings (including other cells) in a number of different ways and many types of chemical forces are involved (2,5,176). In the present context the interest is restricted to interactions that confer selectivity (recognition) and take place at the cell surface (cell surface recognition) (1-5,110).

In many cases of cell surface recognition the mechanism is known to or thought to resemble the binding of antibody to an antigen. For simplicity in such cases the binding agent is termed ligand and the molecule at the cell surface to which it binds is termed receptor (131) (Fig. 3). The ligand is bound to the receptor by weak short-range non-covalent forces such as electrostatic interaction, hydrogen bonds and also hydrophobic interactions induced by a stereochemical fit of a part of the ligand with a part of the receptor (lock-and-key interaction) (177, chapter by Pauling, L. in 1). The latter largely forms the basis for the selectivity of receptor ligand interaction.

Thus, the feature of the cell surface recognized by a ligand is in the first place the presence of a receptor with the appropriate shape and molecular surface properties although the binding also depends on other parameters (concentration, 110,178,179, availability, 110,171, state of the membrane, 110,171, and cytoskeleton, 180). In other cases ligand cell surface recognition may be a more complex process depending on several steps. Of special interest in the present context is the role of carbohydrate chains as receptors as currently discussed (3-5,10,105,110,139,171,181). These may be linked to protein (in glycoproteins) or to lipid (in glycolipids) (Fig. 2). A number of examples for glycosphingolipids are given below.

It has also been postulated that specific cell-cell recognition may occur without a receptor-ligand interaction. For example, it was suggested (182) that cells with similar surface glycoprotein structures may form aggregates "by large numbers of weak non-specific forces" in a process resembling the segregation of large polymers into separate phases. Glycosphingolipids could possibly contribute to such a process as they are major cell surface components and in addition the structure of these major components vary from one cell to another.

6.5. Glycolipids as cell surface recognition markers

The glycosphingolipid patterns vary between different tissues, species and individuals as illustrated above and in Tables II-IV and also between closely related cells such as different classes of lymphocytes (183,184) and epithelial cells from different parts of the intestines (paper IV, 27,185). Therefore, glycolipids are candidates to act as recognition markers characteristic for the tissue and species origin and individuality of cells. This may be as receptors for various types of ligands or other mechanisms (see above).

The best established cases are for ligands of another organism (self - not self interaction). Thus, glycolipids are antigenic (186,187) and act as blood group antigens (70,110,139), heterophil antigens (70,110), autoimmune antigens (125), receptors for cold agglutinins (188), embryonal antigens (110-115), differentiation antigens (110), marker antigens for different lymphocytes (110,121-124,183) and tumour antigens (110,116). Glycosphingolipids may also act as receptors for bacterial toxins (110,171,189,190), virus (190) and the adhesion of whole bacteria (142,191,192, paper V).

Glycolipids are thought to act as receptors for ligands within the organism (self-self interaction) but the physiological occurrence of this remains to be proven, e.g. receptors for hormones (193), macrophage migration inhibitory factor (194) and other agents (110). In cell-cell interaction lectins (Barondes et al. in 4), glycosyltransferases (166) and glycosidases (195) have been suggested as ligands.

In addition, glycolipids have been proposed to interact with small organic molecules (110), e.g. opiates (196) and ions, e.g. K^+ (11) and Ca^{2+} (197).

REFERENCES

1. Landsteïner, K. (1947) The Specificity of Serological Reactions Harvard University Press, Cambridge, Massachusetts.
2. Greaves, M.F. (1976) in Receptors and Recognition (Cuatrecasas, P. & Greaves, M.F., eds.) Vol. 1A, pp. 1-32, Chapman and Hall, London.
3. Beachey, E.H. ed. (1980) Bacterial Adherence, Receptors and Recognition, Ser. B, Vol. 6, Chapman and Hall, London.
4. Barondés, S.H. ed. (1976) Neuronal Recognition, Chapman and Hall, London.
5. Frazier, W. & Glaser, L. (1979) Ann. Rev. Biochem. 48, 491-523.
6. Thudichum, J.L.W. (1884) A treatise on The Chemical Constitution of the Brain, Ballière, London (Facsimile edition (1962) Archon Books, Hamden, Connecticut).
7. Carter, H.E., Glick, F.J., Norris, W.P. & Phillips, G.E. (1947) J. Biol. Chem. 170, 285-294.
8. Macher, B.A. & Sweeley, C.C. (1978) in Complex Carbohydrates, Methods in Enzymology (Ginsburg, V. ed.) Vol. 50, pp. 236-251, Academic Press, New York.
9. Sweeley, C.C. & Siddiqui, B. (1977) in The Glycoconjugates (Horowitz, M.I. & Pigman, W. eds.) Vol. I, pp. 459-540, Academic Press, New York.
10. Nagai, Y. & Yamakawa, T. (1978) Trends Biochem. Sci. 3, 128-131.
11. Karlsson, K.-A. (1977) in Structure of Biological Membranes (Abrahamsson, S. & Pascher, I. eds.) pp. 245-274, Plenum Press, New York.
12. IUPAC-IUB Commission on Biochemical Nomenclature (1977) Eur. J. Biochem. 79, 11-21.
13. IUPAC-IUB Combined Commission on Biochemical Nomenclature (1967) Eur. J. Biochem. 1, 259-266.
14. Kościelak, J., Miller-Podraza, H., Krauze, R. & Piasek, A. (1976) Eur. J. Biochem. 71, 9-18.
15. Gardas, A. (1976) Eur. J. Biochem. 68, 177-183.
16. Dejter-Juszynski, M., Harpaz, N., Flowers, H.M. & Sharon, N. (1978) Eur. J. Biochem. 83, 363-373.
17. Karlsson, K.-A. (1970) Lipids 5, 878-891.
18. Tanford, C. (1973) The Hydrophobic Effect, John Wiley & Sons, New York.
19. Singer, S.J. & Nicolson, G.L. (1972) Science 175, 720-731.
20. Bretscher, M.S. (1973) Science 181, 622-629.
21. Op den Kamp, J.A. (1979) Ann. Rev. Biochem. 48, 47-71.

22. Svanborg-Edén, C., Leffler, H. (1980) Scand. J. Infect. Disease Suppl. 24, 144-147.

23. Yamakawa, T., Suzuki, S. (1952) J. Biochem. 39, 393-402.

24. McKibbin, J.M. (1978) J. Lipid Res. 19, 131-147.

25. Suzuki, C., Makita, A. & Yosizawa, Z. (1968) Arch. Biochem. Biophys. 127, 140-149.

26. Weiser, M. (1973) J. Biol. Chem. 248, 2536-2541.

27. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1980) in Cell Surface Glycolipids (Sweeley, C.C. ed.) pp. 79-104, ACS Symposium Series 128, Amer. Chem. Soc., Washington D.C.

28. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1981) Exp. Cell Res. 135, 1-13.

29. Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H., Pimlott, W. & Samuelsson, B.E. (1978) FEBS Lett. 89, 42-46.

30. Florkin, M. (1973) Comp. Biochem. Physiol. 44B, 1-10.

31. Breimer, M.E., Hansson, G.C. & Karlsson, K.-A. (1981) J. Biol. Chem. in press.

32. Ångström, J., Breimer, M.E., Falk, K.-E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1981) J. Biol. Chem. in press.

33. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H., (1981) J. Biol. Chem. in press.

34. Ångström, J., Breimer, M.E., Falk, K.-E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1981) Arch. Biochem. Biophys. in press.

35. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1980) Biochim. Biophys. Acta 617, 85-96.

36. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H., unpublished.

37. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1980) Biochem. Biophys. Res. Comm. 95, 416-422.

38. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H. (1980) FEBS Lett. 114, 51-56.

39. Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H. Pimlott, W. & Samuelsson, B.E. (1981) FEBS Lett. 124, 299-303.

40. Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H. J. Biol. Chem. in press.

41. Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H. unpublished.

42. Karlsson, K.-A., Samuelsson, B.E. & Steen, G.O. (1973) Biochim. Biophys. Acta 316, 317-335.

43. Karlsson, K.-A. (1976) in Glycolipid Methodology (Witting, C.A. ed.) pp. 97-122, Amer. Oil Chem. Soc., Champaign, Illinois.

44. Karlsson, K.-A. (1978) Progr. Chem. Fats other Lipids 16, 207-230.

45. Falk, K.-E., Karlsson, K.-A. & Samuelsson, B.E. (1979) Arch. Biochem. Biophys. 192, 164-176; 177-190; 191-202.

46. Laine, R.A., Hsieh, T.C.-Y. & Lester, R.L. (1980) in Cell Surface Glycolipids (Sweeley, C. ed.) pp. 65-78, ACS Symposium Series 128, Amer. Chem. Soc., Washington D.C.

47. Itasaka, O. & Hori, T. (1979) J. Biochem. 85, 1469-1481.

48. Ledeen, R.W. & Yu, R.K. (1976) in Glycolipid Methodology (Witting, L.A. ed.) pp. 187-214, Am. Oil. Chem. Soc., Champaign, Illinois.

49. Svennerholm, L. & Fredman, P. (1980) Biochim. Biophys. Acta 617, 97-109.

50. Watanabe, K., Hakomori, S.-i., Powell, M.E. & Yokota, M. (1980) Biochem. Biophys. Res. Comm. 92, 638-646.

51. Lingwood, C.A., Hakomori, S.-i. & Ji, J.H. (1980) FEBS Lett. 112, 265-268.

52. Sonnino, S., Ghidoni, R., Marchesini, S., Tettamanti, G. (1979) J. Neurochem. 33, 177-121.

53. Ghidoni, R., Sonnino, S., Tettamanti, G., Baumann, N., Reuter, G. & Schauer, R. (1980) J. Biol. Chem. 255, 6990-6995.

54. Murray, R.K., Narasimhan, R., Levine, M., Pinteric, L., Shirley, M., Lingwood, C. & Schachter, H. (1980) in Cell Surface Glycolipids /Sweeley, C.C. ed.) pp. 105-125, ACS Symposium Ser. 128, Amer. Chem. Soc., Washington D.C.

55. Sastry, P.S. (1974) Adv. Lipid Res. 12, 251-340.

56. Kates, M. (1970) Adv. Lipid Res. 8, 225-265.

57. Waldi, D. (1962) in Dünnschicht-Chromatographie (Stahl, E. ed.) pp. 496-515, Springer-Verlag, Berlin.

58. Svennerholm, L. (1963) J. Neurochem. 10, 613-623.

59. Breimer, M., Hansson, G., Karlsson, K.-A., Larson, G., Leffler, H., Pascher, I., Pimlott, W. & Samuelsson, B. (1980) in Advances in Mass Spectrometry (Quayle, A. ed.) Vol. 8B, pp. 1097-1108, Heyden, London.

60. Waller, G.R. & Dermer, O.C. eds. (1980) Biochemical Applications of Mass Spectrometry, John Wiley, New York.

61. Karlsson, K.-A. (1970) Chem. Phys. Lipids 5, 6-43.

62. Laine, R.A., Renkonen, O. (1975) J. Lipid. Res. 16, 102-106.

63. Gerwing, G.J., Kamerling, J.P. & Vliegenhart, J.F.G. (1979) Carbohydrate Res. 77, 1-7.

64. Burlingame, A.L., Ballile, T.A., Derrick, P.J. & Chizhov, O.S. (1980) Anal. Chem. 52, 214R-258R.

65. Matsuo, T., Matsuda, H. & Katakuse, I. (1980) in Advances in Mass Spectrometry (Quayle, A. ed.) pp. 990-996, Heyden & Sons, London.

66. Hiramoto, R.N., Smith, E.C., Ghanta, V.K., Shaw, J.F. & McKibbin, J.M. (1973) J. Immunol. 110, 1037-1043.

67. Holmgren, J., Lönnroth, I., Månsson, J.-E. & Svennerholm, L. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2520-2524.

68. Breimer, M.E. & Karlsson, K.-A., unpublished.

69. Karlsson, K.-A. & Larson, G. (1981) FEBS Lett. 128, 71-74.

70. Hakomori, S.-i. & Kobata, A. (1974) in The Antigens (Sela, M. ed.) Vol. II, pp. 79-140, Academic Press, New York.

71. Marcus, D.M., Naiki, M. & Kundu, S.K. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 3263-3267.

72. Kościelak, J., Miller-Podraza, H., Krauze, R. & Cedergren, B. (1976) FEBS Lett. 66, 250-253.

73. Breimer, M.E., Cedergren, B., Karlsson, K.-A., Nilsson, K. & Samuelsson, B.E. (1980) FEBS Lett. 118, 209-211.

74. Zbedska, E., Magnuska, A., Kusnierz, G., Seyfried, H. & Kościelak, J. (1980) FEBS Lett. 120, 33-36.

75. Adams, E.P., Gray, G.M. (1968) Chem. Phys. Lipids 2, 147-155.

76. Yasue, S., Handa, S., Miagawa, S., Inoue, J., Hasegawa, A. & Yamakawa, T. (1978) J. Biochem. 83, 1101-1107.

77. Hamanaka, S., Handa, S., Inoue, J., Hasegawa, A. & Yamakawa, T. (1980) J. Biochem. 87, 639-643.

78. Hamanaka, S., Handa, S. & Yamakawa, T. (1979) J. Biochem. 86, 1623-1626.

79. Hamanaka, S., Handa, S., Inoue, J., Hasegawa, A. & Yamakawa, T. (1979) J. Biochem. 86, 695-698.

80. Nagai, Y. & Iwamori, M. (1980) Mol. Cell Biochem. 29, 81-90.

81. Stellner, K., Saito, H., and Hakomori, S.-i. (1973) Arch. Biochem. Biophys. 155, 464-472.

82. Ohashi, M. & Yamakawa, T. (1973) J. Lipid Res. 14, 698-700.

83. Yamazaki, T., Suzuki, A., Handa, S. & Yamakawa, T. (1979) J. Biochem. 86, 803-809.

84. Macher, B.A. & Klock, J.C. (1980) J. Biol. Chem. 255, 2092-2096.

85. Hansson, G.C., Karlsson, K.-A., Leffler, H. & Strömbäck, N., unpublished.

86. Handa, N. & Handa, S. (1965) Jpn. J. Exp. Med. 35, 331-341.

87. Kornfeld, R., Kornfeld, S. (1980) in The Biochemistry of Glycoproteins and Proteoglycans (Lennarz, W.J. ed.) pp. 1-34, Plenum Press, New York.

88. Dawson, G. (1978) in The Glycoconjugates (Horowitz, M.I. & Pigman, W. eds.) Vol. II, pp. 256-286, Academic Press, New York.

89. Yamakawa, T., Handa, S. & Hamanaka, S. (1979) in Proceedings of the Fifth International Symposium on Glycoconjugates (Schauer, R., Boer, P., Buddecke, E., Kramer, M.F., Vliegenthart, J.F.G. & Wiegandt, H. eds.) pp. 593-594, Georg Thieme Publishers, Stuttgart.

90. Walker, E.P. (1964) Mammals of the world, John Hopkins Press, Baltimore.

91. Breimer, M.E., Karlsson, K.-A. & Samuelsson, B.E. (1981) J. Biol. Chem. in press.

92. Cooper, J.R. & Kent, P.W. (1978) Biochem. Biophys. Res. Comm. 83, 622-627.

93. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. & Leffler, H., unpublished.

94. Ferguson, A. (1980) Biochemical Systematics and Evolution, Blackie, Glasgow.

95. Matsuda, G., Takei, H. & Maita, T. (1974) in Cellular and Molecular Biology of Erythrocytes (Yoshikawa, H. & Rapoport, S.M. eds.) Urban & Schwartzenberg, Berlin.

96. Abelson, J. & Butz, E. eds. (1980) Science 209, 1317-1438.

97. Rushton, A.R. (1975) J. Mol. Evol. 6, 15-37.

98. Weinberg, R.A. (1980) Cell 22, 643-644.

99. Benveniste, R.E., Todaro, G.J. (1974) Nature 252, 456-459.

100. Abrahamsson, S., Dahlén, B., Löfgren, H., Pascher, I. & Sundell, S. (1977) in Structure of Biological Membranes (Abrahamsson, S. & Pascher, I. eds.) pp. 1-23, Plenum Press, New York.

101. Wieslander, Å., Christiansson, A., Rilfors, L. & Lindblom G. (1980) Biochemistry 19, 3650-3655.

102. Falk, K.-E., Karlsson, K.-A. & Samuelsson, B.E. (1980) Chem. Phys. Lipids 27, 9-21.

103. Hansson, G.C., Karlsson, K.-A. & Samuelsson, B.E. (1978) J. Biochem. 83, 813-819.

104. Glickman, R.M. & Bouhours, J.F. (1976) Biochim. Biophys. Acta 424, 17-25.

105. Harrison, F.L. & Chesterton, C.J. (1980) FEBS Lett. 122, 157-165.

106. Nishikimi, M., Udenfriend, S. (1977) Trends Biochem. Sci. 2, 111-113.

107. Jain, H.K. (1980) Nature, 288, 647-648.

108. Marcus, D.M. & Janis, R. (1970) J. Immunol. 104, 1530-1539.

109. Tanaka, N. & Leduc, E.H. (1956) J. Immunol. 77, 198-212.

110. Hakomori, S.-i. (1981) Ann. Rev. Biochem. 50, 733-764.

111. Stern, P.L., Willison, K.R., Lennox, E., Galfré, G., Milstein, C., Secher, D., Ziegler, A. & Springer, T. (1978) Cell 14, 775-783.

112. Kapadia, A., Feizi, T. & Evans, M.J. (1981) Exp. Cell Res. 131, 185-195.

113. Hakomori, S.-i., Nudelman, E., Levery, S., Solter, D. & Knowles, B.B. (1981) Biochem. Biophys. Res. Comm. 100, 1578-1586.

114. Gooi, H.C., Feizi, T., Kapadia, A., Knowles, B.B., Solter, D., Evans, M.J. (1981) Nature 292, 156-158.

115. Stinnakre, M.G., Evans, M.J., Willison, K.R. & Stern, P.L. (1981) J. Embryol. exp. Morph. 16, 117-131.

116. Hakomori, S.-i. & Young, W.W. (1978) Scand. J. Immunol. 7, Suppl. 6, 97-117.

117. Forssman, J. (1911) Biochem. Ztschr. 37, 78-115.

118. Buchbinder, L. (1935) A.M.A. Arch. Path. 19, 841-880.

119. Carlsson, H.-E., Sundblad, G., Hammarström, S., Perlmann, P. & Gustafsson, B.-E. (1978) Arch. Biochem. Biophys. 187, 366-375.

120. Zweibaum, A., Oudea, P., Halpern, B. & Veyre, C. (1966) Nature 209, 159-161.

121. Young, W.W., Hakomori, S.-i., Durdik, J.M. & Henney, C.S. (1980) J. Immunol. 124, 199-201.

122. Schwarting, G.A. & Summers, A. (1980) J. Immunol. 124, 1691-1694.

123. Momoi, R., Wiegandt, H., Arndt, R. & Thiele, H.-G. (1980) J. Immunol. 125, 2496-2500.

124. Nakahara, K., Ohashi, T., Oda, T., Hirano, T., Kasai, M., Okumura, K. & Tada, T. (1980) New Eng. J. Med. 302, 674-677.

125. Hirano, T., Hashimoto, H., Shiokawa, Y., Iwamori, M., Nagai, Y., Kasai, M., Ochiai, Y. & Okumura, K.(1980) J. Clin. Invest. 66, 1437-1440.

126. Kobata, A. (1977) in The Glycoconjugates (Horowitz, M.I. & Pigman, W. eds.) Vol. I, pp. 423-440, Academic Press, London.

127. Karlsson, K.-A. & Larson, G. (1981) J. Biol. Chem. 256, 3512-3524.

128. Messer, M. (1974) Biochem. J. 139, 415-420.

129. Messer, M. & Kerry, K.R. (1973) Science 180, 201-203.

130. Zo Bell, C.E. (1943) J. Bacteriol. 46, 39-56.

131. Jones, G.W. (1977) in Microbial Interactions (Reissig, J.L. ed.) Receptors and Recognition, Ser. B., Vol. 3, pp. 139-146, Chapman and Hall, London.

132. Svanborg-Edén, C., Hagberg, L., Hanson, L.A., Korhonen, T., Leffler, H. & Olling, S. (1981) in Adherence and Microorganism Pathogenicity, Ciba Foundation Symposium 80, Pitman Medical, Tunbridge Wells, pp. 161-187.

133. Gibbons, R.J. & van Houte, J. (1975) Ann. Rev. Microbiol. 29, 19-44.

134. Rutter, J.M., Burrows, R., Sellwood & Gibbons, R.A. (1975) Nature 257, 135-136.

135. Cheeney, C.P., Schad, P.A., Formal, S.B. & Boedeker, E.C. (1980) Infect. Immun. 28, 1019-1027.

136. Svanborg-Edén, C., Hanson, L.A., Jodal, U., Lindberg, U. & Sohl-Åkerlund, A. (1976) Lancet II, 490-492.

137. Kabat, E.A. (1976) Structural Concepts in Immunology and Immunochemistry, Holt Rinehart and Winston, New York.

138. Kabat, E.A. (1978) J. Supramol. Structure 8, 515-524.

139. Watkins, W.M. (1978) Proc. R. Soc. London B202, 31-53.

140. Marcus, D.M., Kundu, S.K. & Suzuki, A. (1981) Seminars in Hematology 18, 63-71.

141. Naiki, M., Fong, J., Ledeen, R. & Marcus, D.M. (1975) Biochemistry 14, 4831-4836.

142. Källenius, G., Möllby, R., Svensson, S.B., Winberg, J., Lundblad, A., Svensson, S. & Cedergren, B. (1980) FEMS Microbiol. Lett. 7, 297-302.

143. Källenius, G., Möllby, R., Hultberg, H., Svensson, S.B., Cedergren, B. & Winberg, J. (1981) Lancet II,604-606.

144. Leffler, H. & Svanborg-Edén, C. (1981) Infect. Immun. in press.

145. Korhonen, T., Leffler, H. & Svanborg-Edén, C. (1981) Infect. Immun. 32, 796-804.

146. Lomberg, H., Jodal. U., Svanborg-Edén, C., Leffler, H. & Samuelsson, B.E. (1981) Lancet I, 551-552.

147. Fletscher, K.S., Bremer, E.G. & Schwarting, G.A. (1979) J. Biol. Chem. 254, 11196-11198.

148. Springer, G.F. (1971) Progr. Allergy 15, 9-77.

149. Marx, J.L. (1980) Science 209, 1103-1106.

150. Stoll, B.J., Bardhan, P.K., Greenough, W.B. III, Holmgren, J., Huq, I., Fredman, P. & Svennerholm, L. (1980) Lancet II, 888-891.

151. Tayot, J.-L., Holmgren, J., Svennerholm, L., Lindblad, M. & Tardy, M. (1981) Eur. J. Biochem. 113, 249-258.

152. Holmgren, J. & Svennerholm, A.-M. (1973) Infect. Immun. 5, 662-667.

153. Slomiany, B.L. & Slomiany, A. (1980) in Cell Surface Glycolipids (Sweeley, C. ed.) pp. 149-176, ACS Symposium Series 128, Amer. Chem. Soc., Washington D.C.

154. Stubbs, C.D., Ketherer, B., Hicks, R.M. (1979) Biochim. Biophys. Acta 558, 58-72.

155. Hakomori, S.-i. (1970) Chem. Phys. Lipids 5, 96-115.

156. Björkman, L.R., Karlsson, K.-A., Pascher, I. & Samuelsson, B.E. (1972) Biochim. Biophys. Acta 270, 260-265.

157. Karlsson, K.-A., Leffler, H. & Samuelsson, B.E. (1979) Biochim. Biophys. Acta 574, 79-93.

158. Sugita, M., Shirai, S., Hasaka, O. & Hori, T. (1975) J. Biochem. 77, 125-130.

159. Araki, S., Komai, Y. & Satake, M. (1980) J. Biochem. 87, 503-510.

160. Kaufman, B., Basu, S. & Roseman, S. (1971) J. Biol. Chem. 246, 4266-4271.

161. Yamamoto, P., Yano, I., Masui, M. & Yaboouchi, E. (1978) J. Biochem. 83, 1213-1216.

162. LaBach, J.P. & White, D.C. (1969) J. Lipid Res. 10, 528-534.

163. Crean, E.V. & Rossomando, E.F. (1979) Arch. Biochem. Biophys. 196, 186-191.

164. Steck, T.L. & Dawson, G. (1974) J. Biol. Chem. 249, 2135-2142.

165. Gahmberg, C.G. & Hakomori, S.-i. (1973) J. Biol. Chem. 248, 4311-4317.

166. Roseman, S. (1970) Chem. Phys. Lipids 5, 270-297.

167. Beyer, T.-A., Sadlar, J.E., Rearick, J.I., Paulson, J.C. & Hill, R.L. (1981) Adv. Enzymology 52, 23-175.

168. Schachter, H. & Roseman, S. (1980) in The Biochemistry of Glycoproteins and Proteoglycons (Lennarz, W.J. ed.) pp. 85-160, Plenum Press, New York.

169. Dawson, G. (1978) in The Glycoconjugates (Horowitz, M.I. & Pigman, W. eds.) Vol. II, pp. 287-336, Academic Press, New York.

170. Kijimoto-Ochiai, S., Naiki, M. & Makita, A. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5407-5410.

171. Critchley, D.R. & Vicker, M.G.(1977) in Dynamic Aspects of Cell Surface Organization (Poste, G., & Nicholson, G.C. eds.)

172. Fishman, P.H. & Henneberry, R.C. (1980) in Cell Surface Glycolipids (Sweeley, C.C. ed.) pp. 223-239, ACS Symposium Series 128, Amer. Chem. Soc., Washington D.C.

173. Chatterjee, S. & Sweeley, C.C. (1973) Biochem. Biophys. Res. Comm. 53, 1310-1316.

174. Coles, L., Hay, J.B. & Gray, G.M. (1970) J. Lipid Res. 11, 158-163.

175. Hay, J.B. & Gray, G.M. (1970) Biochim. Biophys. Acta 202, 566-568.

176. Curtis, A.S.G. (1967) The Cell Surface: Its Molecular Role in Morphogenesis, Logos Press (Academic Press), London.

177. Fischer, E. (1894) Ber. Deut. Chem. Ges. 27, 2985

178. Fishmann, P.H. & Atikkan, E.E. (1979) J. Biol. Chem. 254, 4342-4344.

179. Tsai, C.-M., Zopf, D.A. & Ginsburg, V. (1978) Biochem. Biophys. Res. Comm. 80, 905-910.

180. Rees, D.A., Lloyd, C.W. & Thom, D. (1977) Nature 267, 124-128.

181. Hughes, R.C. (1975) in Essays in Biochemistry (Campbell, P.N. & Aldridge, W.N. eds.) Vol. II, pp. 1-36, Academic Press, New York.

182. Edwards, P.A.W. (1978) Nature 271, 248-249.

183. Stein, K.E. & Marcus, D.M. (1977) Biochemistry 16, 5285-5291.

184. Rosenfelder, G., Herbst, H. & Braun, D.G. (1980) FEBS Lett. 114, 213-218.

185. Hansson, G.C., Karlsson, K.-A. & Thurin, J. (1980) Biochim. Biophys. Acta 620, 270-280.

186. Rapport, M.M. & Graf, L. (1969) Progr. Allergy 13, 273-331.

187. Marcus, D.M. & Schwarting, G.A. (1976) Adv. Immunol. 23, 203-240.

188. Feizi, T., Childs, R.A., Watanabe, K. & Hakomori, S.-i. (1979) J. Exp. Med. 149, 975-980.

189. van Heyningen, W.E. (1974) Nature 249, 415-417.

190. Holmgren, J., Elwing, H., Fredman, P., Strannegård, Ö. & Svennerholm, L. (1980) Adv. Exp. Biol. Med. 125, 453-470.

191. Kearns, M.J. & Gibbons, R.R. (1979) FEMS Microbiol. Lett. 6, 165-168.

192. Faris, A., Lindahl, M. & Wadström, T. (1980) FEMS Microbiol. Lett. 7, 265-269.

193. Kohn, D.L., Consiglio, E., DeWolf, M.J.S., Grollman, E.F., Ledley, F.D., Lee, G. & Morris, N.P. (1980) Adv. Exp. Med. Biol. 125, 487-503.

194. Miura, T., Handa, S. & Yamakawa, T. (1979) J. Biochem. 86, 773-776.

195. Rauvala, H., Carter, W.G. & Hakomori, S.-i. (1981) J. Cell Biol. 88, 127-137.

196. Loh, H.H., Law, P.Y., Ostwald, T., Cho, T.M. & Way, L.E. (1978) Fed. Proceedings 37, 147-152.

197. Jaques, L.W., Brown, E.B., Barrett, J.M., Brey, W.S. & Wellner, W. (1977) J. Biol. Chem. 252, 4533-4538.

198. Hakomori, S.-i. (1981) Seminars in Hematology 18, 39-62.

199. Hanada, E., Handa, S., Kunio, K. & Yamakawa, T. (1978) J. Biochem. 83, 85-90.

200. Hanfland, P., Egge, H., Dabrowski, U., Kuhn, S., Roelcke, D., Dabrowski, J. (1981) Biochemistry 20, 5310-5319.

201. Gray, G.M., White, R.J. & Wajer, J.R. (1978) Biochim. Biophys. Acta 528, 127-137.

202. Watanabe, K., Powell, M.E. & Hakomori, S.-i. (1979) J. Biol. Chem. 254, 8223-8229.

203. Chien, J.-L. & Hogan, E.L. (1980) in Cell Surface Glycolipids (Sweeley, C.C. ed.) pp. 135-148, ACS Symposium Series 128, Washington D.C.

204. Rauvala, H., Krusius, T. & Finne, J. (1978) Biochim. Biophys. Acta 531, 266-274.

205. Iwamori, M. & Nagai, Y. (1978) J. Biol. Chem. 253, 8328-8331.

206. Watanabe, K. & Hakomori, S.-i. (1979) Biochemistry 182, 5502-5504.

207. Tadano, K. & Ishizuka, I. (1980) Biochem. Biophys. Res. Comm. 97, 126-132.

208. Slomiany, B.C. & Slomiany, A. (1978) J. Biol. Chem. 253, 3517-3520.

209. Slomiany, A., Kojima, K., Banas-Gruszka, Z. & Slomiany, B.L. (1981) Biochem. Biophys. Res. Comm. 100, 778-784.

210. Watanabe, K., Powell, M., Hakomori, S.-i. (1978) J. Biol. Chem. 253, 8962-8967.

211. Sonnino, S., Ghidoni, R., Galli, G. & Tettamanti, G. (1978) J. Neurochem. 31, 947-956.

212. Rauvala, H. (1976) FEBS Lett. 62, 161-164.

213. Forstner, G.G. & Wherrett, J.R. (1973) Biochim. Biophys. Acta 306, 446-459.

ISBN 91-7222-447-9